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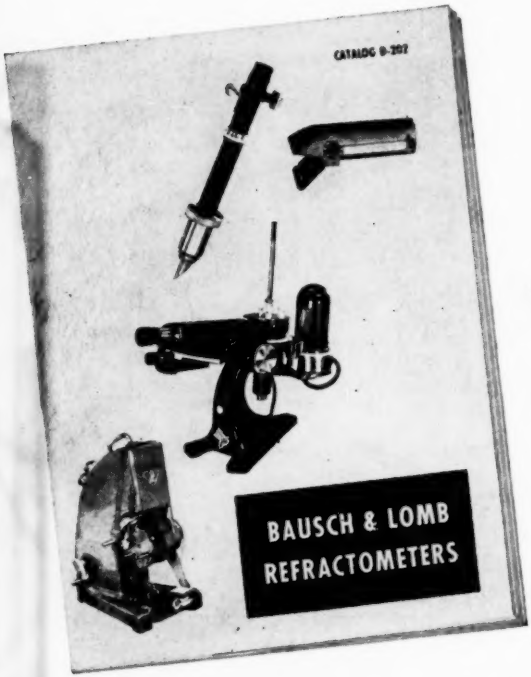

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THE ROLE OF TEMPERATURE IN AMINO ACID LOSSES DURING THE PROLONGED STORAGE OF EVAPORATED MILK

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(Manuscript received May 29, 1952)

In an earlier study *(4)* it was shown that on prolonged storage of 5 years at room temperature there is a significant loss of lysine, histidine, and arginine from evaporated milk. It has also been shown that small amounts of lysine and histidine are lost during the sterilization of evaporated milk *(3)*. Browning, probably of the Maillard reaction type, occurs during both sterilization and storage, and the rate of browning during storage is related to temperature as well as time. These facts suggest storage losses of amino acids might be minimized by cold storage and accelerated by high storage temperatures. The investigation to be reported was undertaken to confirm this suggestion.

EXPERIMENTAL

The storage conditions were selected to give a wide variation in storage temperature rather than to reflect practical storage conditions. However of the three storage temperatures chosen, the lowest approximates the conditions found in an evaporated milk cold storage warehouse, the variable intermediate temperature approximates that found on a grocer's shelf and the highest represents extreme storage conditions probably seldom met in commercial practice. Samples of 5 days of commercial production of evaporated milk were taken and divided into three lots. One lot was stored in an electric refrigerator at 40°F.(4°C.). A second lot was stored at room temperature under temperature conditions which usually varied between 75° and 84°F.(24° and 29°C.), seldom fell below 68°F.(20°C.), and occasionally rose to 95°F.(35°C.). A third lot was stored in a hot room at approximately 100°F.(38°C.). Other samples were hydrolyzed and assayed for the 10 "essential" amino acids immediately. At the end of one year and two years, samples were taken from storage, hydrolyzed and analyzed. The methods of hydrolysis and assay used were those of Stokes, Gunness, Dwyer, and Caswell *(8)*.

In addition to the amino acid analyses, the proteins of the evaporated milk stored at the 3 temperatures were evaluated by the rat growth method. For this work a storage period of 27 months was used since unavoidable delays prevented the investigation being started at the end of 2 years. Here 5 weanling rats per group were used and the experiment was continued for 6 weeks. The rats were fed a ration prepared by dissolving dextrose in the evaporated milk to give a mixture with a protein content of 9% on a dry basis. This mixture was supplemented with 125 γ of thiamine, 1 mg. of calcium pantothenate, 1 mg. of niacin amide, 250 γ of riboflavin, 100 γ of pyridoxine, 40 γ of biotin, 40 γ of folic acid, 40 U.S.P. Units of vitamin A, 5 U.S.P. Units of vitamin D, 3 mg. of alpha-tocopherol, 100 mg. of choline chloride, and 500 mg. of Jones and Foster *(6)* mineral mixture per 100 g. dry ration. The vitamin supplement is based on the vitamin requirements of the rat as stated by Brown and Sturtevant *(1)*. Liberal quantities of minerals and certain vitamins are also supplied by the evaporated milk. The feeding was ad libitum but daily individual records of food consumption were kept. The rats were housed in individual cages and weighed weekly. The positions of the cages in the animal room were rotated daily by groups to equalize temperature variations caused by nearness to windows, heaters, etc.

RESULTS

The results of the storage checks are summarized in Table 1. The difference in percent of the original value and the standard error of the mean are given for each amino acid. It should be stated that the standard errors and the "t" values which may be calculated from them include some error between assays, as well as the storage loss. In order to estimate the amount of error which occurs between assays because of differences in the standard curve and the standard curve to sample relationship, the percent difference for series of 4 or 5 samples assayed at different times but without significant storage differences were calculated for 12 series of samples. The

TABLE 1

Loss of amino acids during storage of evaporated milk at three different temperatures

Amino acid	Average percent amino acid in evaporated milk (original content)	Average percent change after 1 year of storage at			Average percent change after 2 years of storage at		
		40°F.[a]	Room temperature[b]	100°F.[c]	40°F.[a]	Room temperature[c]	100°F.[d]
Arginine	0.213	0±2.10	+1±0.70	−11±2.50	0±2.23	−3±1.11	−28±0.86
Histidine	0.166	−1±1.87	−2±1.32	−13±2.50	0±0.70	−5±0.86	−29±2.54
Isoleucine	0.481	−2±0.70	−1±1.00	− 1±1.41	+1±1.32	+1±0.50	0±0.70
Leucine	0.667	+1±0.83	+2±1.00	+ 1±2.25	−1±0.50	−1±0.86	− 2±0.70
Lysine	0.481	+2±1.73	−2±1.93	−10±2.87	−1±2.82	−8±3.60	−29±2.82
Phenylalanine	0.330	−3±1.00	−2±1.50	− 2±1.34	+1±1.11	+1±0.50	+ 1±1.11
Threonine	0.308	−2±0.60	−2±0.45	− 2±2.35	+1±1.22	0±1.11	0±0.70
Tryptophane	0.091	−4±2.18	−4±1.11	− 7±1.41	0±1.73	−1±1.00	−12±1.58
Valine	0.428	+2±2.05	−3±0.83	+ 2±2.09	+2±1.00	+2±0.70	+ 2±0.70

[a] Appearance and palatability of the samples was good.
[b] Appearance and palatability of the samples had decreased.
[c] Appearance and palatability of the samples had markedly decreased. Possibly they would no longer be acceptable for all the customary uses of evaporated milk.
[d] Samples no longer acceptable as human food.

average difference was 2.2% and the range was from 0.0 to 6.2%. For the comparisons presented in this report it appears that differences should be greater than 5% and also more than 2.78 times the standard error (t is 2.78) in order for a storage loss to be indicated. With these criteria no losses of essential" amino acids are indicated at 40°F.(4°C.) or room temperature, but losses of tryptophane, lysine, histidine, and arginine are indicated at the 100°F.(38°C.) storage temperature during the 1-year storage period.

At the end of the second year no losses are indicated for the samples stored at 40°F.(4°C.). In confirmation of earlier work *(4)* slight losses of lysine and histidine are indicated at room temperature storage. Large losses of tryptophane, 12%; lysine, 29%; histidine, 29%; and arginine, 28% are indicated at 100°F.(38°C.) storage.

The study reported here was concerned primarily with nutritive value and no attempt was made to collect extensive data on appearance and palatability. In the footnotes to Table 1 general statements concerning the acceptability of the samples will be found. The limitations of the observations recorded there should be recognized. These statements are based on the opinion of one or more judges rather than extensive taste panel

tests or on physical measurements of color, degree of separation, etc. The acceptability of evaporated milk varies with taste preference of the individual and the use for which the product is intended. A sample rejected as a beverage might be highly acceptable for making candy or for cooking with other foods having a strong characteristic flavor. Webb, Deysher, and Potter *(9)* have studied the changes which occur in the physical condition of evaporated milk during storage at a number of different temperatures. They found that commercial evaporated milk of heavy body remained acceptable for 2 years when held in undisturbed storage below 60°F.(15.5°C.). Physical deterioration was rapid during storage above 70°F.(21°C.).

No data for methionine are given in Table 1. At the end of the first year of storage, suspicion had arisen that the methionine method being used was not sufficiently accurate for the problem under investigation. At that time two assays were made, the first was too high, the second was too low, however the average agreed fairly well with the earlier value even though the variability of the data was high. At the end of the second year of storage two microbiological assays were conducted. Both assays gave results averaging higher than obtained originally and the values were extremely erratic. Only one conclusion could be reached. The data were of no value for comparative purposes. Although it was no longer possible at the end of the 2-year period to repeat the investigation, the opportunity for checking the comparative value of the methionine content of the samples stored at three temperatures still existed. This comparison was made using *L. citrovorum* according to the method outlined by Steele, Sauberlich, Reynolds, and Bauman *(7)* and a fresh series of hydrolysates. The results of this assay were much more uniform than those with *S. faecalis*. The average methionine content was 0.215 ± 0.007%. When the methionine content of the sample stored at 40°F.(4°C.) was taken as 100, the comparative values for the samples stored at room temperature showed an average of 1 ± 1.58% loss and those for the samples stored at 100°F.(38°C.) an average of 5 ± 2.06% loss. The difference in retention between storage temperatures does not appear to be marked. The average methionine content of the milk proteins for the 5 cold storage samples was 3.20%. Compared to many values found in the literature and determined in our laboratory, this is a high value for the methionine content of milk proteins. In the absence of complete storage data, this fact, together with evidence presented earlier *(4)* suggests no storage loss of methionine occurs at the lower temperatures.

The results of the rat growth experiment evaluating the proteins of the evaporated milk stored at each of the 3 temperatures for 27 months are especially interesting. During the 6-week trial the rats fed the milk stored at 40°F.(4°C.) gained an average of 52.4 g., those fed the milk stored at room temperature gained an average of 26.0 g. and those fed the milk stored at 100°F.(38°C.) gained —0.8 g. An analysis of variance shows that the differences caused by the storage temperature are significant. (F is 52.6, and the 99% table value of F is 6.93). As a measure of protein efficieney the grams gain in weight per gram protein fed were calculated

for each group. For the group fed the proteins from the evaporated milk stored at 40°F.(4°C.) this figure was 2.19, for the group fed the proteins from the milk stored at room temperature it was 1.40, and for the group fed the proteins from the milk stored at 100°F.(38°C.) it was —0.06. Analysis of variance shows that the variations introduced by the storage temperature of the milk is highly significant (F is 82.9, and the 99% table value for F is 6.93).

In a rat growth experiment such as that just described there may be some question as to whether protein is the deficiency limiting growth. In an effort to further answer this question, the group fed the ration prepared from the milk stored at 100°F.(38°C.) were continued an additional week on the same ration and in addition were offered a supplement of purified casein. The rats ate the supplement very poorly but at the end of a week they showed an average gain of 10 g. This is a small but remarkable difference, since during the preceding 6 weeks they had shown an average net gain of —0.8 g. These data further verify our conclusion that it is a protein change during storage that is being measured by the rat growth tests.

DISCUSSION

Webb, Deysher, and Potter *(9)* have shown the effects of storage temperature on the properties of evaporated milk. It has been shown by Whitnah *(10)*, Cook, Weast, and Parker *(2)*, and Hodson *(4, 5)* that no significant loss of protein nutritive value or essential amino acids occurs at room temperatures during the customary storage period for evaporated milk. The losses of nutritive value which occur during prolonged storage at high temperatures are appreciable but not as striking as the changes in palatability and appearance that occur simultaneously. A sample stored for 2 years at 40°F.(4°C.) is very similar to a recently processed sample. A sample that has been stored for 2 years at room temperature has lost much of its consumer appeal. A sample stored for 2 years at 100°F. (38°C.) is not acceptable as food. Such a sample would not be found on the retail market. The acceptability and nutritive value of evaporated milk is directly related to the time and temperature of storage. It is the practice in the industry to build up inventories of evaporated milk during the flush season of milk production. These stocks are held in cold storage at 40°F.(4°C.) until released to the trade during the succeeding winter months. These marketing practices do not detract from the nutritive value, attractiveness, or palatability of the product.

SUMMARY

Evaporated milk was stored for 2 years at 40°F.(4°C.), room temperature, and 100°F.(38°C.). At the end of the first year of storage no losses of amino acids were found at 40°F.(4°C.) or room temperature, but measurable losses of tryptophane, lysine, histidine, and arginine were found at 100°F.(38°C.). After 2 years of storage there were no losses of amino acids at 40°F.(4°C.), slight losses of lysine and histidine at room temperature and large losses of tryptophane, 12%; lysine, 29%; histidine, 29%; and arginine, 28% at 100°F.(38°C.). When the proteins from evaporated milk stored for 27 months at the 3 temperatures were compared by the rat

growth method, the proteins from the milk stored at 40°F.(4°C.) were superior to those from milk stored at room temperature, and the latter were very superior to those stored at 100°F.(38°C.). These changes in amino acid content and protein nutritive value while striking are not as important as the detrimental changes in appearance and palatability which occur simultaneously and preclude the marketing of a product where such changes have occurred.

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THE OCCURRENCE OF TRIMETHYLAMINE AND TRIMETHYLAMINE OXIDE IN FRESH WATER FISHES[a]

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(Manuscript received August 1, 1951)

There appears in the literature conflicting reports concerning the occurrence of trimethylamine (TM) and trimethylamine oxide (TMO) in fresh water fish. Many workers *(1, 2)* state that fresh water fish do not contain (TMO). However, there are workers *(4, 8, 11)* who have reported that some fresh water fish do contain (TMO) and/or (TM). It has been noted that various fresh water fish caught in western Massachusetts, develop a fishy odor suggestive of (TM). There are no data to substantiate the occurrence of (TM) or (TMO) in fresh water fish from this area. The nearest area from which data have been reported is Canada. Data from different geographical locations differ regarding the (TMO) content of marine fish. Perhaps the same variation exists with fresh water fish. No explanation has been found to account for the variation in (TM) among different species of marine fish, nor among the same species of marine fish in different waters. It is thought that these variations exist because of the feed of the fish. In this work, several species of fresh water fish were examined for (TMO) and (TM) content. To observe the effect of a feed containing (TMO), fresh water trout fed a diet containing relatively large amounts of (TMO) and (TM) were examined.

EXPERIMENTAL

Fresh water fish were caught, brought back to the laboratory alive, and held in frozen storage until analyzed. The species taken in nearby waters were small mouth bass *(Micropterus dolomieu)*, pickerel *(Esox niger)*, white perch *(Morone americana)*, yellow perch *(Perca flavescens)*, black crappie *(Pomoxis nigor-maculatus)*, sunfish *(Lepomis gibbosus)*, blue gill *(Lepomis m. machrochirus)*, Eastern golden shiner *(Notemigonus c. crysoleucas)*, bullhead *(Ameirurus sp.)* and Brown trout *(Salmo trutta fario)*. All species except the trout were taken during the summer months from Lake Lashaway, East Brookfield, Massachusetts. Trout roe and trout ranging in age from six months to five years were obtained from the Massachusetts State Trout Hatchery at Sunderland.

All fish were examined for (TM) and (TMO) by the method described by Ronald and Jakobsen *(8)*. Only muscle tissue was used for examination.

RESULTS

The (TM) and (TMO) content of the species examined are shown in Table 1. The species which were found to contain (TMO) were small mouth bass, pickerel, black crappie, white perch, sunfish, and blue gill. The amounts of oxide detected were small when compared to the oxide content of various marine fish. In the case of the small bony fish such as sunfish, blue gill, and yellow perch, several fish were filleted, and the fillets ground and mixed in order to contain a sufficiently large sample. The figures of 17.1 milligrams per cent for the blue gill and 5.6 milligrams

[a]Contribution No. 867 from the Massachusetts Agricultural Experiment Station.

TABLE 1

The trimethylamine oxide and trimethylamine content of the muscle of different species of fresh water fishes

Species	Trimethylamine oxide-N, mg.%	Trimethylamine-N, mg.%
Small mouth bass *(Micropterus dolomieu)*	4.8	4.4
Pickerel *(Esox niger)*	11.0	1.8
White perch *(morone americana)*	12.0	0.8
Yellow perch *(Perca flurescens)*	Undetectable	0.6
Black crappie *(Pomoxis nigro-maculatus)*	14.6	2.0
Sunfish *(Lepomis gibbosus)*	5.6	4.8
Blue gill *(Lepomis m. machrochirus)*	17.1	4.0
Eastern golden shiner *(Notemigonus c. crysoleucas)*	Undetectable	3.0
Bullhead *(Ameiurus sp.)*	Undetectable	0.6

per cent for the sunfish represent an average of three samples obtained from over 20 of each species. With the larger fish, each specimen was analyzed separately, and the results from several individuals of the same relative size were averaged. With white perch it was found that the small fish examined contained only traces of the oxide, while the large fish contained an average of 11 milligrams percent. The bass analyzed were very small (four to five inches). It would be interesting to see if the flesh of larger bass contained an increased amount of oxide.

The (TM) and (TMO) content of the different aged hatchery trout are shown in Table 2. In considering these results, the diet of these trout

TABLE 2

The trimethylamine oxide and trimethylamine content of hatchery raised brown trout (Salmo trutta fario) at different ages

Age of fish	Trimethylamine oxide-N, mg.%	Trimethylamine-N, mg.%
Roe	Undetectable	Undetectable
Six months	Undetectable	1.7
Two years	7.5	4.5
Four years	16.0	2.9
Five years	18.5	0.9

is important. According to Norrell *(7)*, it is difficult to say just how much a hatchery trout is fed per day. The amount fed usually varied from 3 to 10% of the body weight of the fish. The fish were on a diet which was found to be (TMO)- and (TM)-free until they were about eight months old. After this time, the fish were fed a mixed ration containing herring bits. This mixed feed contained approximately 15 milligrams percent (TMO) and 4 milligrams percent (TM). Table 2 shows that in fish up to six months of age, (TMO) was not detected. It was found that as the age of the fish increased, the (TMO) content increased, while the (TM) content decreased. This is difficult to explain unless the (TMO) becomes less susceptible to loss through decreased enzymic action or for some other reason.

DISCUSSION

Information available on the feeding habits of the fresh water fish is not complete enough to permit a study of the correlation of feeding habits

to the data obtained on the (TMO) and (TM) content of the fish examined. Kapeller-Adler and Vering *(5)* reported that fresh water plants and plankton do not contain (TMO). Therefore, it seems unlikely in the present study that the (TMO) is of exogenous origin. Grollman *(3)* postulated that (TM) results from protein metabolism. Tarr *(10)* demonstrated that in human subjects there is a large amount of (TMO) secreted which is of endogenous origin. It is difficult to arrive at a definite conclusion as to the origin of (TMO) in fresh water fish.

From the conditions under which (TMO) was found in the hatchery trout, it appears evident that in this case the origin of the oxide was exogenous. This is in agreement with the findings of Benoit and Norris who concluded from feeding tests with young salmon, reported in 1945, that at least in the case of salmon, the occurrence of (TMO) was due to the accumulation of ingested (TMO) rather than to endogenous metabolic function.

SUMMARY AND CONCLUSIONS

Small mouth bass, pickerel, white perch, yellow perch, black crappie, sunfish, blue gills, Eastern golden shiners, and bullheads, were analyzed for trimethylamine oxide and trimethylamine. All species were found to contain small amounts of trimethylamine. The small mouth bass, pickerel, white perch, black crappie, sunfish, and blue gill were found to contain trimethylamine oxide. These amounts were small in comparison to marine fish.

The trimethylamine oxide content of hatchery raised brown trout, fed a diet containing trimethylamine oxide from herring, increased with age of fish, and appeared to be of exogenous origin. On the other hand, trimethylamine in the other fresh water fish apparently originated in the muscle trimethylamine oxide.

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MAPLE SIRUP. IV. EFFECT OF HEATING SIRUPS UNDER CONDITIONS OF HIGH TEMPERATURE AND LOW WATER CONTENT: SOME PHYSICAL AND CHEMICAL CHANGES

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In previous publications *(3, 5)* and in a patent *(6)*, it was stated that additional maple flavor and increased color can be developed in a commercial maple sirup by heating it at the elevated boiling temperatures obtained by atmospheric evaporation of the sirup to a sufficiently high solids content. This observation was based on the following experimental facts: (1) sirups of normal flavor and color are made from normal sap by atmospheric evaporation to a solids content of 65-67%; (2) concentration of normal sap by freeze drying produces a colorless and flavorless sirup, proving that normal sap is devoid of flavor and color; (3) maple flavor and the usual amber color can be produced in this colorless and flavorless sirup by heating at its boiling point. It was also shown that a high-flavored sirup produced by atmospheric evaporation of water from a normal sirup until the solids content reached 85-90% and holding at the elevated boiling point of the more concentrated sirup, had much less caramel and other off-flavors than was produced by heating sirups in an autoclave for identical times and temperatures where the solids were kept at 65.5% *(5)*. No data on quantitative changes in the heated sirup were given, nor were any data given showing the effect of time and temperature on the flavor and color of the heated sirup.

The data presented in this paper indicate some of the changes that take place under the different heating conditions.

MATERIALS, METHODS, AND PROCEDURE

Materials. The sirups were of two commercial table grades, Fancy (U. S., New York, or Vermont Grade) and New York Grade No. 3 (Vermont Grade C). These grades were chosen because they represent the extremes of the commercially available sirup. Previous work demonstrated that data for intermediate grades of sirup will fall between the data obtained with these two sirups.

Equipment. The sirups were heated in small, stainless steel, steam kettles which were two-thirds jacketed and which could be operated with steam pressures up to 40 p.s.i. One gallon of sirup more than filled the jacketed portion of this small kettle. An electric stirrer (600 r.p.m.) was used to keep the thickened sirup in motion and prevent super-heating at the surfaces of the kettle. To minimize further evaporation, a tight-fitting, stainless steel lid was placed on the kettle when the desired boiling point was reached.

Methods and procedure. The samples of sirup were defecated with lead and deleaded with potassium oxalate. Schoorl's method *(1)* was used to determine free invert sugar and reducing sugars after inversion with hydrochloric acid at room temperature.

Color was evaluated in terms of light-absorption measurements at 450 mμ in the General Electric recording spectrophotometer, with water as a blank, and a solution

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diluted to give observed absorbance (optical density) in the range 0.2 to 0.8. From this measurement, a color index was calculated as the absorbance at 450 mμ for a 1-cm. depth of solution having 86.3 gms. of solids per 100 ml. of solution.

This color index $= A_{1\,cm.}^{86.3\%} = A_{450}$ (86.3/bc), where A_{450} is the observed absorbance at 450 mμ, b is the depth of the solution in centimeters (1 cm. in these experiments), and c is the grams of solids as sucrose per 100 ml. of solution, as determined on an Abbé refractometer. The maximum color indices of commercial sirups for the three U. S. Standard table grades are: U. S. AA (Light Amber), 0.506; U. S. A (Medium Amber), 0.900; and U. S. B (Dark Amber), 1.47.

Ultraviolet absorption measurements were made on a Cary recording spectrophotometer, with the same solutions against water as a blank. From these data, absorptivities were calculated.

Hydrogen ion concentrations (pH) were determined with glass electrodes on aliquots of the diluted samples used in the spectrophotometric studies.

One gallon of the sirup was placed in the steam kettle and heated with 35 p.s.i. steam, bringing the sirup to boiling as rapidly as possible. Vigorous boiling was continued to remove sufficient water to elevate the boiling point to the desired temperature. The kettle lid was set in place and the steam pressure reduced sufficiently to hold the concentrated sirup at a simmering boil at the temperature being investigated. This temperature was maintained constantly throughout the heating period. When samples were removed for analysis, however, the boiling equilibrium was often upset, resulting in an increase of about 2°F. for a short period until the boiling equilibrium could be re-established by addition of a small amount of water.

A sample of the original sirup was analyzed for (a) pH, (b) absorption at 450 mμ, (c) general absorption in the ultraviolet region of the spectrum, (d) invert sugar, and (e) reducing sugars after inversion by the methods given above. Samples of the cooking sirup were taken when the sirup first reached the temperature being studied, which was designated as zero time. Other samples were taken at half-hour intervals until the material was no longer usable because of off-flavors. Each sample of heated sirup was adjusted to the same refractive index (solids content) as that of the original sirup by the addition of distilled water so that they would be on a comparable basis. If the boiling point began to rise because of incomplete reflux, distilled water was added to the sirup to return the boiling point to that being studied. The temperatures used were 235°, 244°, and 252°F. for the two grades of sirup. The approximate water content of the sirups at these boiling temperatures were 20, 15, and 10%, respectively *(4)*.

RESULTS AND DISCUSSION

Table 1 gives data on changes in the pH, color index at 450 mμ, the percentage of invert sugar, and flavor that occurred in the two grades of sirup heated at the three temperatures for the periods indicated. Flavor level was determined by dilution of the sample with colorless and flavorless cane sugar sirup (having a refractive index the same as the original maple sirup) required to yield a flavor equivalent to that of the original maple sirup. Thus, a 1 + 3 dilution means that 1 volume of the sample was diluted with 3 volumes of cane sugar sirup and indicates a fourfold increase in flavor over the original sirup or a flavor enhancement of 4. A small group of conditioned testers was used to determine flavor levels.

Figure 1 shows the effect on the invert sugar content of time of cooking at each of the three temperatures. Figure 2 shows the effect on the color, in terms of absorption at 450 mμ, of time of cooking. Figure 3 shows the ultraviolet absorption spectrum obtained with the No. 3 sirup before and after it was cooked for 6 hours at 252°F.

Prolonged heating of the Fancy grade sirup resulted in a decrease in pH, amounting to approximately 1.5 units. The same treatment of No. 3 sirup lowered the pH less than 0.2 unit. These changes in pH are signifi-

TABLE 1

Analyses of maple sirups heated for different times at various temperatures above the normal boiling temperature

Heating time	Fancy grade				No. 3 grade			
	pH	Invert sugar content	Color index $A_{1\,cm.}^{66.2\%}$	Flavor enhancement factor[1]	pH	Invert sugar content	Color index $A_{1\,cm.}^{66.2\%}$	Flavor enhancement factor[1]
Boiling temperature, 235°F.								
Hours		%				%		
Orig.	7.0	0.31	0.30		6.7	1.74	1.91	
0	7.0	.31	.39		6.6	1.81	2.23	
0.5	7.0	.34	.52		6.7	1.96	2.43	
1.0	6.8	.45	.62		6.6	2.16	2.81	
1.5	6.7	.53	.76		6.6	2.46	3.06	
2.0	6.6	.61	.89	3	6.6	2.80	3.34	
2.5	6.4	.69	1.03	4	6.6	3.20	3.71	
3.0	6.8	.82	1.16	5	6.6	3.64	3.99	
3.5	6.1	.94	1.32	6	6.6	4.32	4.28	
4.0	6.0	1.08	1.46		6.6	4.99	4.63	
5.0	5.9	1.43	1.87		6.6	6.77	5.37	
6.0	5.8	2.16	2.28		6.6	9.05	6.11	3
Boiling temperature, 244°F.								
Orig.	7.0	0.31	0.30		6.7	1.74	1.91	
0	6.7	.33	.44		6.6	1.88	2.49	
0.5	6.7	.41	.61		6.6	2.11	2.98	
1.0	6.5	.54	.82	3	6.6	2.42	3.40	
1.5	6.3	.72	1.02	3	6.6	2.94	3.89	
2.0	5.7	.92	1.25	4	6.6	3.35	4.31	
2.5	5.6	1.19	1.51	5	6.6	4.28	4.79	
3.0	5.4	1.56	1.78		6.6	5.21	5.27	
3.5	5.5	2.04	2.09		6.6	6.32	5.87	
4.0	5.5	2.59	2.43		6.6	7.72	6.43	3
5.0	5.4	4.15	3.33		6.5	10.95	7.72	4
6.0	5.4	6.14	4.27		6.5	15.50	9.25	6
Boiling temperature, 252°F.								
Orig.	7.0	0.31	0.30		6.7	1.74	1.91	
0	6.6	.46	.69		6.9	2.10	2.89	
0.5	6.1	.66	1.01	3	6.8	2.50	3.55	
1.0	6.0	.95	1.36	4	6.7	3.17	4.18	
1.5	5.7	1.31	1.74	5	6.7	4.11	4.86	
2.0	5.4	1.93	2.19	7	6.6	5.31	5.58	
2.5	5.5	2.59	2.67		6.6	7.17	6.39	3
3.0	5.4	3.36	3.18		6.6	9.15	7.26	4
3.5	5.4	4.40	3.94		6.6	11.01	8.27	5
4.0	5.0	5.97	4.62		6.6	14.29	9.58	
5.0	5.0	9.21	6.25		6.6	21.52	12.95	
6.0	5.0	13.81	8.45		6.6	31.45	17.61	

[1]Flavor enhancement factor of the high-flavored sirup is the ratio of the volume of a blend (1 part of high-flavored sirup + x parts of cane sugar sirup) to one volume of original sirup, both of identical flavor level.

cant because several runs on different sirups gave substantially the same results. The cause of these changes is not fully known. Paper chromatograms of the organic acids from the heated sirups indicated that no change occurred in the number of acids present. However, the color materials, which are acidic and do not migrate to any extent on paper chromato-

grams, did show a relatively large increase. The increase in these acidic substances was detected during the isolation of the organic acid fraction by means of ion-exchange. The amount of anion exchange material required to prevent break-through for a given weight of sample was much larger for the cooked, colored sample than for the uncooked sample. This increase in acidic substances might account for the decrease in pH.

Invert sugar was produced by hydrolysis of the sucrose at all the temperatures studied. The rate of its production was highest at 252°F. and in the No. 3 sirup (Figure 1). Analysis for total reducing sugars after inversion showed that but little sugar was destroyed in the heat-treated sirups except in the No. 3 sirup when heated at the highest temperature (252°F.), in which case a 2% decrease in total sugars was observed. In all cases, the increase in invert sugar was accompanied by a corresponding loss of sucrose, indicating that the amount of sugar involved in the darkening process, either by caramelization or by browning, was small and that the tinctorial power of the coloring materials was high. The rate of invert sugar production approximated a log function.

As indicated in Figure 2, the rate of color development increased as the time of heating increased. The similarity in the shapes of the curves for invert sugar formation and color formation suggests a correlation between these two variables. An evaluation of the data, however, indicates that no direct or simple relationship exists. This fact suggests that the production of color may be related to the formation of some intermediate products from the thermal decomposition of the reducing sugars, and possibly that the reaction of these sugar decomposition products with

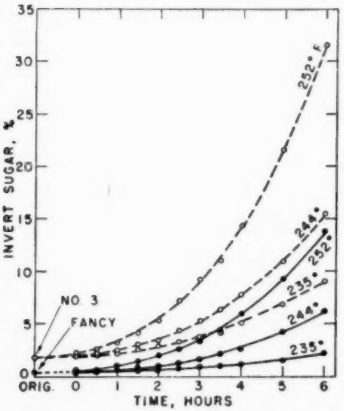

Figure 1. Development of invert sugar in two commercial grades of maple sirup heated at 235°, 244°, and 252°F. for different periods of time under atmospheric pressure.

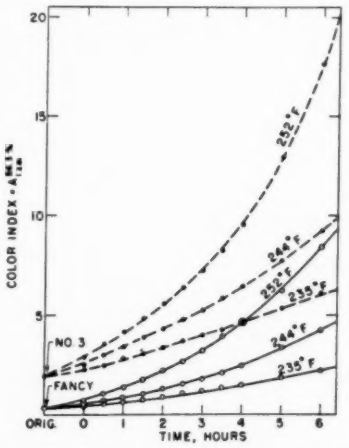

Figure 2. Color index at 450 mμ of two commercial grades of maple sirup heated at 235°, 244°, and 252°F. for different periods of time under atmospheric pressure.

other sirup constituents, as in a browning reaction *(2, 8)*, is responsible for the developed color.

Both grades of sirup darkened during the heating, but the rate of color development, as measured by absorption at 450 mμ was greater at the higher boiling temperatures. It was also greater with the darker grade of sirup than with the lighter grade. Paradoxically, less time was required for a fourfold increase in color index for the Fancy sirup than for the No. 3 sirup (Table 1). Since Fancy sirup is light in color (low color index), the amount of the color bodies that must be produced by the high-flavored process to give a fourfold increase is less than that required for a fourfold increase of color bodies in a No. 3 sirup. However, to develop the same color in a Fancy sirup as in a No. 3 sirup heated for a specified time, the lighter (Fancy) sirup would require a much longer heating treatment.

Interpretation of the ultraviolet absorption spectra for these samples (Figure 3) indicated the production of a conjugated unsaturated material,

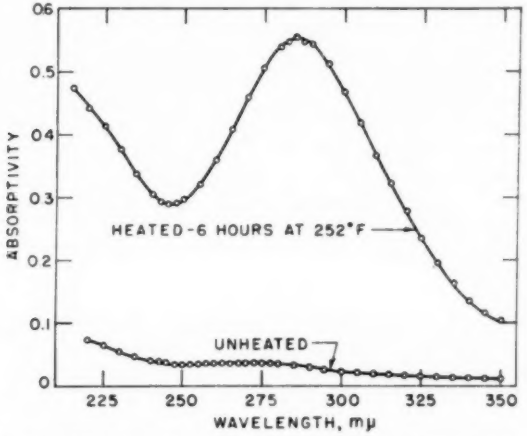

Figure 3. Ultraviolet absorption spectrum of maple sirup (commercial grade No. 3) before and after heating the sirup for 6 hours at 252°F. under atmospheric pressure. Absorptivity is equal to absorbance referred to unit cell length (1 cm.) and unit solids concentration (1 gm./liter).

with an absorption maximum at 285 mμ, the production rate of which followed closely the rate of color development as measured by absorption at 450 mμ. The wave-length of the absorption maximum suggested that the material might be 5-hydroxymethyl-2-furfuraldehyde. Extraction with ethyl acetate of the sirup cooked for 6 hours produced an extract that was insoluble in water *(8)*. The 2,4-dinitrophenylhydrazone derivative of this extract was prepared. This derivative could not be purified by recrystallization from hydrous ethanol solutions and it was necessary to affect purification by chromatographic adsorption on cellulose powder, with ether as

the solvent. An ether solution of the impure hydrazone obtained after recrystallization was placed on a cellulose powder column,[2] and elution was carried out with anhydrous ether. The impurities were preferentially adsorbed, and the derivative was passed through the column in the effluent, from which it was crystallized by slow evaporation of the solvent. Both direct and mixed melting points (197-199° C., corrected) showed that these crystals were identical with those of the derivative made from pure 5-hydroxymethyl-2-furfuraldehyde. Based on a molar absorptivity (a_m) of 16,500 *(7)*, the maximum quantity of 5-hydroxymethyl-2-furfuraldehyde which could be present was 0.4%. It should not be assumed, however, that the hydroxymethylfurfural was responsible for the darkening, since it might have been an end-product of the decomposition of some of the sugars and not an intermediate that reacted to form the coloring material in question. Further study to determine the identity of the coloring materials and to obtain a knowledge of their source is necessary for complete understanding of formation of color in maple sirup.

The increase in maple flavor paralleled the increase in color up to the point where caramel flavor completely masked this more delicate flavor. In general, the data indicate this interdependency (Table 1). If the original color of a sample was darkened fourfold, a fourfold dilution with cane sirup produced a blend having a flavor closely approaching that of the original sirup. This is important, since the absorption of light at 450 mμ can be used to determine the time necessary to reach a predetermined flavor level, eliminating the need for dilution with cane sugar sirup. This method is not applicable, however, if the off-flavors in the cooked sirup are sufficient to mask the maple flavor in the blend. Until the caramel flavor becomes overwhelmingly strong, however, blending with cane sirup appears to dilute the caramel flavor at a greater rate than it dilutes the maple flavor.

SUMMARY

Heating maple sirups with a water content of about 10% at elevated temperatures resulted in the following:

The sirups darkened in color. The rate of formation of color increased with time and temperature of heating, being greatest in the lower grades of sirup.

Development of maple flavor and color closely paralleled one another until the caramel and other off-flavors produced completely masked the more delicate maple flavor.

The content of invert sugar increased with time and temperature of heating. The rate of increase was greater in the darker sirup.

There was no simple relationship between the invert sugar content of the sirups and the rate of formation of color, suggesting that the role of intermediates should be more fully investigated.

The presence of 5-hydroxymethyl-2-furfuraldehyde was proved. The amount of this material increased with time and temperature of heating.

[2] Cellulose powder was made by hammer milling Whatman Ashless Filter Paper Tablets. This was packed in a 1-cm. diameter tube from a suspension in ether. (Mention of commercial products does not imply endorsement by the Department of Agriculture over those of a similar nature not mentioned.)

This material may have no relationship to color but may be the end-product of another reaction.

Acknowledgment

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MAPLE SIRUP. V. FORMATION OF COLOR DURING EVAPORATION OF MAPLE SAP TO SIRUP

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In grading maple sirup, color is one of the principal factors considered, and processors (wholesalers) ordinarily pay a premium price for the lighter colored sirups. Development of a high flavored sirup *(7)*, which can be used as the base of a premium maple sirup blend, has increased the potential demand for light-colored sirups of the higher grades. Under normal conditions, production of light-colored sirup is an indication of the efficiency of the sirup making equipment and of the care and skill used by the producer. Even with the best equipment, however, there are times when the sirup is dark. For these reasons, any information useful in making sirup of improved quality and of light color under all conditions is of extreme value. Many factors contribute to production of color. Hayward and Pederson *(4)* discussed the effect of acid and alkali in the evaporation of maple sap. These authors, as well as Edson, Jones, and Carpenter *(3)*, observed that bacterial growth in the sap often contributed to the production of dark-colored sirup. In addition, factors such as the weather, composition of the sap, and type and condition of the evaporator have definite effects on formation of color.

In the production of high-flavored sirup *(5, 7)*, it was observed that the rate of color development was highest in the more concentrated sirup heated at temperatures above its normal boiling point. This logically led to an investigation of the relationship both of sugar concentration and of the time of heating to formation of color during evaporation of sap to sirup. Such information is essential for basic chemical engineering investigations of evaporator design. The studies reported here indicate the changes in color that may occur in the sap and sirup pans of a maple sirup evaporator during commercial sirup-making.

MATERIALS, METHODS, AND PROCEDURE

Materials. Maple sap used in these studies was obtained by diluting a colorless and flavorless maple sirup to the desired sugar concentration. This sirup was prepared by vacuum evaporation of a fresh, sound maple sap at temperatures below 40°C. The sap had been collected in sterilized equipment from fresh tap holes and spouts and held at temperatures below 35°F.(1.7°C.) until evaporated, usually less than 8 hours after collection. This vacuum-evaporated sirup was essentially colorless and had no detectable flavor. The reason for using colorless sirup is discussed later.

Equipment. All the heating experiments were carried out in a 1-liter, 3-neck Florence flask equipped with two West condensers; one was for refluxing the steam and the other for introducing the glass stirrer. The third neck served both to hold the thermometer and to permit removal of samples for analysis. The flask was heated with a hemispherical heating mantle controlled by a 5-ampere variable autotransformer.

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Methods. Refractive index measurements were made at 20°C. with an Abbé refractometer. Measurements of pH were made with the glass electrode and line-operated pH meter. Absorption measurements at 450 mμ were made on the heated samples without further dilution; the General Electric[b] recording spectrophotometer was used. All other samples except the one having 3.7% sucrose were diluted before analysis to give observed absorbance in the range 0.2 to 0.8. After the sirup was defecated with lead and deleaded with potassium oxalate, invert sugar analyses were made by Schoorl's method *(2)*.

Procedure. The colorless and flavorless sirup was diluted to obtain solutions of the desired solids concentration. The solutions were then degassed by running a thin film of the solution over the inner surface of an evacuated glass sphere. Six hundred milliliters of the degassed sirup solution were placed in the reaction flask and heated to boiling as rapidly as possible, with continuous stirring to obtain uniformity of temperature and to keep foaming at a minimum. A sample, designated *original*, was taken for analysis before heating was started; another, designated *zero time*, was withdrawn when the sirup reached the rolling boil. Thereafter, samples were withdrawn at 15-, 30-, 60-, and 120-minute intervals. All samples were cooled and filtered through glass wool. These samples were analyzed for pH, invert sugar, and absorption at 450 mμ; the results were computed on a solids basis as sucrose, as determined by refractive index measurements at 20°C.

DISCUSSION AND RESULTS

Studies of the effect of time of heating maple sap of different sugar solids content on the formation of color have been hampered in the past by the difficulty of compensating for the color developed during the heating necessary for concentration of the sap (dilute sugar solution) to the desired solids concentration. Also, the effects of time and solids concentration on formation of color in sap heated in commercial evaporators could not readily be determined because of the inhomogeneity of the sap solution throughout the evaporator and lack of means for measuring the time that any particular portion of the sap had been heated. An essentially colorless maple sirup produced by low temperature vacuum evaporation provided a starting material that could be adjusted to the desired solids content with distilled water without development of color, thereby eliminating these problems.

A condition that could not be eliminated, however, was the time required to heat the different sugar solutions to their boiling points. But, because of the shortness of this time, the amount of color developed was so small and so much the same, irrespective of the solids concentration, that this factor was considered insignificant. The effect of the slight trace of color in the original sirup was minimized by subtracting the initial color index[c] of each diluted solution from the color index of the solution heated for the different periods.

The data in Table 1 show the changes that occur in boiling sap of different solids concentrations. Owing to loss of water as steam, each time the flask was unstoppered for removal of samples, the percentage of sol-

[b] Mention of commercial products does not imply endorsement by the Department of Agriculture over others of similar nature not mentioned.

[c] Color index, $A_{1\,cm.}^{66.5\%} = A_{450}$ (86.3/bc), where A_{450} is the observed absorbance at 450 mμ, b is the depth of solution in centimeters (1 cm. in these experiments), and c is the grams of solids as sucrose per 100 ml. as determined on an Abbé refractometer *(5)*.

TABLE 1

Analysis of maple saps of various concentrations of solids heated for different periods of time

Time of heating (minutes)	% solids (sucrose)[a]	Elevation of boiling point °F.[b]	pH	% invert sugar	Color index $A_{1cm.}^{1\%-2\%}$
Original	3.7		5.2	0.17	0
0	3.8		5.2	.17	0.055
15	3.8	0.1	5.0	.16	.229
30	3.8		4.8	.16	.380
60	3.8		4.8	.16	.495
120	3.8		4.7	.16	.646
Original	15.0		6.3	0.60	0
0	15.0		6.0	.59	0.027
15	15.1	0.5	5.9	.59	.166
30	15.5		5.6	.57	.358
60	15.8		5.5	.58	.471
120	15.9		5.5	.59	.585
Original	25.3		6.8	1.15	0
0	25.4		6.8	1.14	0.048
15	25.4	0.8	6.6	1.15	.222
30	25.5		6.3	1.13	.368
60	25.7		6.1	1.17	.483
120	26.0		5.8	1.10	.599
Original	45.5		6.2	2.35	0
0	45.9		6.3	2.19	0.027
15	45.9	2.6	6.4	2.20	.197
30	45.9		6.0	2.23	.401
60	46.1		5.8	2.27	.488
120	46.1		5.7	2.32	.635
Original	50.5		6.2	.....	0
0	50.5		6.2	.....	0.045
15	50.7	3.3	6.0	.....	.292
30	50.7		6.0	.....	.580
60	50.4		5.8	.....	.814
120	50.4		5.6	.....	.853
Original	60.2		7.4	2.77	0
0	60.4		7.0	2.75	0.045
15	60.4	5.1	6.8	2.62	.257
30	60.7		6.5	2.57	.609
60	60.8		6.2	2.62	.828
120	61.3		5.8	2.77	.924

[a] Calculated from the refractive index at 20°C.
[b] Literature reference 6.

ids calculated as sucrose increased slightly during each experiment. This slight increase was negligible because the solutions studied differed so widely in concentration of sucrose.

The saps were degassed to prevent excessive foaming. This removal of dissolved carbon dioxide caused an increase in pH, but since the degree of degassing could not be controlled, the pH of each of the diluted sirups was not identical.

As boiling continued, the acidic materials formed caused a gradual decrease in pH *(1, 5)*. The pH of all the heated sugar solutions except the one with 3.7% solids dropped to about the same value regardless of the initial pH, indicating that loss in carbon dioxide and other possible buffering materials is approximately balanced by production of acids.

The invert sugar content was not affected by heating the sugar solutions for different periods of time. This is compatible with data obtained from studies on heated maple sirups of much higher sugar concentrations. These studies have shown that in the high-flavor process *(5)* heating Fancy sirup at a boiling temperature of 235°F.(113°C.) causes comparatively little increase in invert sugar.

The color of all the solutions increased but not in proportion to the time of heating, as shown in Figure 1. There was little difference in the rate of color formation at solids concentrations from 3.7 to 45.5%. Above this percentage, however, the amount of solids caused a significant increase in the rate of color formation. The greatest rate of increase was noted in the first 30 minutes of heating at the boiling point, irrespective of the solids concentration.

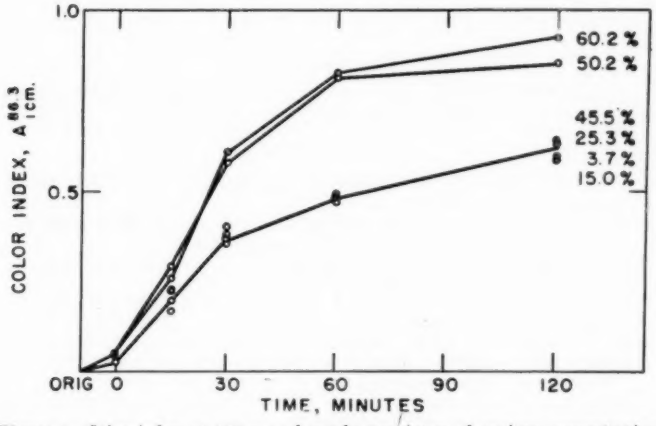

Figure 1. Color index at 450 mμ of maple sap-sirups of various concentrations of sugar heated at their boiling points for different periods of time.

Since color development, in maple sap concentrates up to 45% solids, is independent of the concentration of solids but is a function of the time of boiling, any color developed in the boiling sap in which the solids do not exceed 20-25%, is due only to the time of heating at its boiling temperature. At solids concentrations above 40%, the rate of color development is a function of both time of boiling and concentration of solids. Since the rate is greatest in the more concentrated solution, the efficiency of the evaporating process in the sirup pan is more critical than that in the sap pan. Any areas of localized heating or slow rate of sirup flow in the sirup pan will contribute to dark-colored sirup.

These color data also indicate that the practice of filling a pan with sap and leaving it over a bed of coals for indefinite periods, overnight or longer, between sirup making operations is to be avoided. If it is necessary to suspend operations, the pans should be drained; if the fires are to be banked the sap should be replaced with water.

These observations apply only to fresh unfermented sap. Because of many factors *(3, 4)*, sap contaminated by bacteria produces color at a greatly increased rate, and it is not comparable with the sap used in these experiments.

SUMMARY

Heating at the boiling point maple sap of different solids concentrations, prepared by dilution from vacuum-concentrated colorless and flavorless maple sirup, resulted in the following:

The pH decreased with time of heating, but it was not affected by concentration of solids. The invert sugar content remained constant irrespective of the concentration of solids and the time of boiling. The rate of color development was independent of the solids concentrations up to 45%, but above this percentage there was a significant increase in the rate.

Emphasis must be placed on the design of the evaporator so that sap can be evaporated to sirup in the shortest possible time.

Acknowledgment

The authors wish to express their thanks to Patricia Harper and Mildred Gaspar for their assistance in carrying out this work.

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AUTOSTERILIZATION OF THERMOPHILIC SPORES IN CANNED FOODS[a]

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The study reported here was undertaken to investigate a phenomenon reported to this laboratory by a canner who regularly checked his non-acid vegetable packs for thermophilic spores by incubating samples immediately after processing. Lots of canned peas which showed flat sours in samples incubated at 131°F.(55°C.) when packed, failed to develop any spoilage when tested several months later prior to shipping. Because of the general high resistance of thermophilic spores to destruction by heat, it had not previously been suspected that they might die out during relatively short periods of holding under conditions unfavorable for germination.

A preliminary, inoculated experimental pack of cream style corn was made in 1944, and the results are included here as a matter of interest. Curran and Evans *(1)*, who later made a similar study which included milk and other nutrient and non-nutrient substrates, have referred to this unpublished data in their paper. An experiment with more variables was undertaken the following year, but the samples were destroyed by a laboratory fire. There has now been opportunity to complete work covering several strains of thermophilic bacteria processed at varying levels of sterilizing value in substrates of peas, corn, and dog food.

The term "autosterilization" has frequently been applied to the condition existing in spoiled food where no viable organisms can be recovered by cultural methods, and self-destruction of the spoilage organisms by their own metabolic products is assumed. It is used here in a somewhat different sense to describe the dying out, or loss of viability, of spoilage spores which have never grown in the product.

EXPERIMENTAL

The general plan of the experiments consisted of preparing inoculated packs in enamel-lined thermal death time cans (208×006) for storage, followed by incubation of portions of the samples at selected intervals. The products were packed in the cans using a 13-g. fill, 0.1 ml. of a spore suspension of the desired organism mixed into the contents, and the cans closed under 20 to 22 inches vacuum. The concentration of the spore suspensions used was adjusted to have adequate resistance for surviving the maximum process to be employed. The closed cans were processed at 250°F.(121°C.), except in Pack No. 3 where 260°F.(127°C.) processes were also used. In Packs 2 and 3, three processing times were used at the temperatures employed. All processing times at 250°F.(121°C.) shown in the data have been corrected for heat penetration lag in the thermal death time cans, so that they are also the sterilizing value (F_o) in effective minutes at 250°F.(121°C.). At the conclusion of the process, the cans were immediately cooled.

[a]Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Michigan, June 11, 1952.

The cans were stored at a controlled temperature of 70°F.(21°C.). Immediately after packing, and at selected intervals of storage, a portion of the samples of each lot were incubated for three weeks at 131°F.(55°C.). At the conclusion of the incubation period, each sample was examined for spoilage. Cans inoculated with thermophilic anaerobes were checked for swelling. Cans inoculated with flat sour bacteria were examined by first testing the product for gross sourness with brom-cresol-purple indicator and then by determining the exact pH with a glass electrode pH meter.

Pack No. 1. This exploratory pack was made with cream style corn obtained ready for filling from a commercial cannery. The inoculum used was a spore suspension of organism No. 5A, a thermophilic flat sour bacterium of high heat resistance isolated from cream style corn during a plant survey for thermophilic spoilage hazards. Uninoculated controls were also prepared. Two processes were used, 20.1 minutes at 250°F. (121°C.) and 27.1 minutes at 250°F., which were calculated, respectively, to be substantially below and to approximate the thermal death time of the spore suspension employed. Incubation tests were conducted immediately after packing, and after 6 weeks, 3, 5, and 8 months of storage.

Pack No. 2. The second pack included peas, cream style corn, and dog food. Frozen peas were packed in a 2% salt brine. The cream style corn was prepared in the laboratory from frozen whole kernels. Formulated dog food ready for filling was obtained from a commercial packer.

Different lots of each product were inoculated with spore suspensions of three organisms: 1, National Canners Association No. 1518, a thermophilic flat sour bacterium; 2, No. 5A, the thermophilic flat sour used in Pack No. 1; and 3, No. 99Q, a thermophilic anaerobic bacterium. A fourth lot of uninoculated controls was also prepared for each product. Spore survival counts after the processes used were also run for organism No. 1518, using neutral phosphate buffer in pyrex thermal death time tubes.

The lots of each product were subdivided and processed for 3, 8, and 13 minutes at 250°F.(121°C.) for lots inoculated with the flat sour organisms, and for 3, 7, and 11 minutes for lots inoculated with the thermophilic anaerobe. Incubation tests were conducted over a period of 12 months, tests being made with 8 cans per lot after 0, 1, 4, 8, and 12 months of storage on the inoculated lots. The uninoculated lots were incubated only immediately after packing.

Pack No. 3. In the third pack only cream style corn and inoculation with one organism was used to explore the effect of higher processing temperatures in promoting autosterilization of the spores during storage. As before the product was prepared from frozen whole kernels. National Canners Association No. 1518 thermophilic flat sour was the inoculating organism. Uninoculated controls were prepared for each process.

The processing schedules were 5, 8, and 12 minutes at 250°F., and calculated equivalent times at 260°F.(127°C.) of 1 minute 23 seconds, 2 minutes 13 seconds, and 3 minutes 20 seconds. To check the equivalency of the schedules at the two temperatures, spore survival counts were run on samples from each lot after processing.

Since the survival counts indicated that the 260°F. processes were too long, the pack was made again with shorter process times at 260°F. The calculated sterilizing value of these processes (F_o) is shown in Table 5.

The two parts of this pack were given incubation tests after 0, 2, 6, 10, and 14 or 15 months of storage. In this case 15 cans of each lot were tested at each storage interval.

RESULTS

Results obtained in Pack No. 1 are shown in Table 1. It will be noted that with the lower process where all samples incubated at 131°F.(55°C.) immediately after packing became flat sour, the percentage of sour cans decreased with increasing storage period. After 8 months of storage, the incubation test yielded no flat sour cans. With the higher process, the number of surviving spores was apparently much lower so that even immediate incubation did not result in the souring of all samples. Rapid autosterilization is noticeable in this lot with only one sour can developing

at the 6-week test and none after 3 months, although one additional sour "skip" did show up at 6 months.

The results of Pack No. 2 are presented in Tables 2, 3, and 4 which show the results with the cream style corn, the peas, and the dog food, respec-

TABLE 1

Flat sour spoilage in cream style corn on incubation testing after various storage intervals (Pack No. 1)

Lot No.	Inoculum	Process	No. cans incubated[b]	Incubated cans sour after storage period of				
				None	6 weeks	3 months	6 months	8 months
		minutes at 250°F.		%	%	%	%	%
1	5A	20.1	16	100	56	25	31	0
2	5A	27.1	17	70	6	0	6	0
3	None	20.1	10	0	....	....	....	....
4	None	27.1	10	0	....	....	....	....

[b]At each storage interval.

TABLE 2

Spoilage in cream style corn on incubation testing after various storage intervals (Pack No. 2)

Lot No.	Inoculum	Process	Incubated cans spoiled after storage period of				
			None	1 month	4 months	8 months	12 months
		minutes at 250°F.	%	%	%	%	%
1	1518 flat sour	3	100	100	100	100	100
2	1518 flat sour	8	100	100	62	75	0
3	1518 flat sour	13	100	75	0	0	0
4	5A flat sour	3	100	100	100	100	100
5	5A flat sour	8	100	100	100	100	87
6	5A flat sour	13	100	62	0	0	0
7	None	3	100[c]	....	....	....	....
8	None	8	12[c]	....	....	....	....
9	None	13	0	....	....	....	....
10	99Q thermophilic anaerobe	3	100	100	100	100	100
11	99Q thermophilic anaerobe	7	50	87	62	100	37
12	99Q thermophilic anaerobe	11	0	12	25	0	0

[c]Flat sour spoilage.

tively. The numbers of spores surviving of flat sour No. 1518 when processed in neutral phosphate buffer were as follows:

Process	Average spore count
minutes at 250 F.	*ml.*
3	612.0
8	34.0
13	0.5

TABLE 3

Spoilage in peas on incubation testing after various storage intervals (Pack No. 2)

Lot No.	Inoculum	Process	Incubated cans spoiled after storage period of				
			None	1 month	4 months	8 months	12 months
		minutes at 250°F.	%	%	%	%	%
13	1518 flat sour	3	100	100	100	87	75
14	1518 flat sour	8	100	100	87	75	0
15	1518 flat sour	13	100	100	25	0	0
16	5A flat sour	3	100	100	100	100	100
17	5A flat sour	8	100	100	100	100	75
18	5A flat sour	13	100	37	37	0	0
19	None	3	0	....	....	....	....
20	None	8	0	....	....	....	....
21	None	13	0	....	....	....	....
22	99Q thermophilic anaerobe	3	100	100	100	100	100
23	99Q thermophilic anaerobe	7	75	100	62	100	100
24	99Q thermophilic anaerobe	11	25	37	12	100	12

TABLE 4

Spoilage in dog food on incubation testing after various storage intervals (Pack No. 2)

Lot No.	Inoculum	Process	Incubated cans spoiled after storage period of				
			None	1 month	4 months	8 months	12 months
		minutes at 250°F.	%	%	%	%	%
25	1518 flat sour	3	100	12	0	0	0
26	1518 flat sour	8	12	0	0	0	0
27	1518 flat sour	13	0	0	0	0	0
28	5A flat sour	3	75	25	0	0	0
29	5A flat sour	8	0	0	0	0	0
30	5A flat sour	13	0	0	0	0	0
31	None	3	0	....	....	....	....
32	None	8	0	....	....	....	....
33	None	13	0	....	....	....	....
34	99Q thermophilic anaerobe	3	100	100	100	100	100
35	99Q thermophilic anaerobe	7	100	100	100	100	87
36	99Q thermophilic anaerobe	11	37	100	100	100	0
37	None	3	12[d]	....	....	....	....
38	None	7	0	....	....	....	....
39	None	11	0	....	....	....	....

[d] One swelled can.

Looking first at the results obtained when flat sour organisms were used as the inoculum, it will be noted that with cream style corn and peas, all of the samples given all processes soured when incubated immediately after packing. The corn had a light natural flat sour flora sufficient to survive the 3 minutes at 250°F.(121°C.) process, but which was almost eliminated by processing 8 minutes at 250°F. At the lowest process of

3 minutes, viable spores remained in the peas and corn throughout the 12-month storage period resulting in total, or nearly so, spoilage in the samples incubated. The same was true for the 8-minute process with inoculum 5A, but with No. 1518 some drop in the amount of spoilage was shown after 4 and 8 months of storage with no spoilage after 12 months. With the 13-minute process a more rapid loss of spore viability was found for both organisms, no spoilage developing in the corn after 4 months of storage and in the peas after 8 months of storage.

The flat sour organisms in dog food showed much less retention of viability in this substrate, and yielded sour cans immediately after processing in only the lower processes. Even here the number of sour cans dropped off rapidly with no sour cans being found after 4 months of storage.

When thermophilic anaerobe No. 99Q was used as the inoculum, it will be seen from Tables 2, 3, and 4 that no consistent reduction in swells was obtained with any of the product substrates upon incubation testing after the several storage intervals. The longer processes tended to reduce the percentage of cans spoiling, especially at the most severe process. However, even with this process which was apparently close to the thermal resistance of the inoculum, varying amounts of spoilage developed throughout the storage period.

The data from Pack No. 3 are given in Table 5. Although the 260°F. (127°C.) processes used in the first section of the pack were calculated to be equivalent to the 250°F.(121°C.) cooks, the spore survival counts show that they possessed considerably greater lethality. In the second section of the pack where reduced 260°F. processes were employed with a more concentrated inoculum, comparable destruction of spores was still not obtained. At both processing temperatures, the lower process times which allowed substantial numbers of spores to survive gave high percentages of flat sour cans with reduction after the longer storage intervals. As in the previous packs, Lots such as 3, 8, 9, 8A, and 9A which received the longer processes in relation to the inoculum used, show early reductions in the number of sour cans after storage as contrasted to substantially complete spoilage at the time of packing. Most of these lots were free from flat sours after 10 months of storage, some after 2 or 6 months.

DISCUSSION

The results presented show definitely that thermophilic flat sour bacterial spores lose viability when stored in non-acid food substrates at 70°F.(21°C.), a temperature below their growth range. All of the experimental packs illustrate that the extent and rapidity of loss of viability is closely related to the sterilizing value of the process used. This in turn governs the number of spores surviving in the cans, as shown by the counts in Table 5. Attempts to determine the effect of increasing the processing temperature, retaining the same sterilizing value, were not entirely successful because of lack of equivalent lethality in the processing schedules used. However, there is some indication that use of higher temperature promotes autosterilization of the spores during storage. For example in Table 5, Lots 2 and 7 have similar numbers of surviving spores, but Lot 7 processed at 260°F. shows the earlier onset of autosterilization and it is

TABLE 5

Flat sour spoilage in cream style corn on incubation testing after various storage intervals (Pack No. 3)

Lot No.	Inoculum	Process				No. spores surviving per can	Incubated cans sour after storage period of				
		Minutes	Seconds	Temperature	F_0		None	2 months	6 months	10 months	14 or 15 months*
				°F.			%	%	%	%	%
1	1518	5	0	250	5	32,500	100	100	100	93	93
2	1518	8	0	250	8	9,945	100	100	86	60	60
3	1518	12	0	250	12	390	100	100	26	6	6
4	None	5	0	250	5	.....	6	....	....	....	....
5	None	8	0	250	8	.....	0	....	....	....	....
6	None	12	0	250	12	.....	0	....	....	....	....
7	1518	1	23	260	5	11,400	100	86	26	13	0
8	1518	2	13	260	8	97	86	13	6	0	0
9	1518	3	20	260	12	5	86	0	0	0	0
10	None	1	23	260	5	.....	13	....	....	....	....
11	None	2	13	260	8	.....	0	....	....	....	....
12	None	3	20	260	12	.....	0	....	....	....	....
1A	1518	5	0	250	5	126,360	100	100	100	93	86
2A	1518	8	0	250	8	19,500	100	100	100	66	60
3A	1518	12	0	250	12	Lost	100	100	100	46	0
4A	None	5	0	250	5	.....	0	....	....	....	....
7A	1518	0	51	260	3	55,900	100	100	93	73	60
8A	1518	1	21	260	4.8	3,848	100	93	20	0	0
9A	1518	2	5	260	7.5	Lost	100	80	0	0	0
12A	None	2	5	260	7.5	.....	0	....	....	....	....

*Lots 1 to 12, 15 months of storage; Lots 1A to 12A, 14 months of storage.

complete at 14 months when Lot 2 still gave 60% flat sours. Again, Lot 7A, Table 5, processed at 260°F. shows a similar amount of autosterilization to Lot 2A processed at 250°F., although the spore survival count in Lot 7A is roughly three times greater.

The reason for this loss of viability by flat sour spores is not clear. Microscopic examination has failed to give any visible evidence of physical changes in the spore cell structure. The phenomenon is undoubtedly related to the heat shock imparted to the spores since unprocessed spore suspensions retain their viability over long periods of time. Just what physical and physicochemical changes are induced in the spores that cause them to gradually lose the ability to germinate in favorable substrate is not known at this time. Curran and Evans *(1)* discuss in some detail possible mechanisms by which the heat shock that activates spores of this type for ready germination may lead to self-destruction of the cell.

The results given in Tables 2, 3, and 4 show that a typical thermophilic anaerobe strain is not subject to this autosterilization of the spores during storage of the food product. It must be assumed, therefore, that of the two most prevalent types of thermophilic bacteria, only the flat sour species can be expected to lose spore viability while stored at temperatures below the growth range. Fortunately, the thermophilic anaerobes generally do not run to the extremely high heat resistances found in many flat sour species. Therefore, careful attention to reducing the number of thermophilic anaerobic spores in the product as much as possible enables the usual canned food processes to control spoilage from this source.

The fact of autosterilization of thermophilic flat sour spores established by the work reported here, together with some of the conditions which influence it, may be of considerable practical importance to canners in their programs to control flat sour spoilage. Since the canning of most vegetables and many other non-acid foods requires heat treatments during preparation, it is often extremely difficult to exclude all flat sour spores from the product. Good cooling practices and cool warehousing can prevent any spoilage while the goods are under the canner's control. However, the product may sour after being shipped out because of high storage temperatures at some point in distribution channels. This is a serious problem for the Armed Forces who must ship canned foods into all climates, frequently for storage under makeshift conditions. Even in domestic distribution too many instances of flat sour spoilage occur from storage in poorly constructed warehouses in the warmer areas or storage near heaters, steam lines, and the like.

Applying the results of this study, danger of flat sours occurring in distribution could be alleviated if not entirely eliminated by 1, reducing the number of flat sour spores in the product entering the can as much as practical; 2, using a process of the highest sterilizing value compatible with quality, preferably at the highest processing temperature suitable to conditions; and 3, storage at cool warehouse temperatures for several months. The number of spores surviving in the can after processing, which was found to govern the rate and completeness of their autosterilization, is dependent on many factors, including heat resistance of the strain, numbers originally in the product, and lethality of the process used. Therefore,

it is not possible to specify a time of storage for complete autosterilization except under definite known conditions. A practical method of determining the status of any one lot with respect to viable flat sour spores might consist of incubating samples at 131°F.(55°C.) immediately after packing, and again at suitable intervals during the storage period.

SUMMARY

Commercial observation that canned peas containing spores of thermophilic flat sour bacteria were no longer subject to spoilage when incubated at 131°F.(55°C.) after several months of warehouse storage, was investigated by laboratory inoculated packs. Cream style corn, peas, and dog food were packed in thermal death time cans, inoculated with spore suspensions of two different flat sour strains and one thermophilic anaerobe, processed at different levels of sterilizing value, and stored at 70°F.(21°C.). At intervals samples were removed, incubated at 131°F.(55°C.) and examined for spoilage.

It was found that the thermophilic flat sour spores undergo autosterilization in these products during storage at temperatures below their growth range, while the thermophilic anaerobic spores were not subject to this action. With the flat sour organisms, increasing the sterilizing value of the process which reduced the number of surviving spores, increased the rate and completeness of autosterilization. There was some indication that increasing the processing temperature also promoted loss of spore viability.

Practical application of these findings can be made to reduce the danger of flat sours occurring during distribution when canned foods may be subjected to warm storage. After reducing the number of spores surviving in the product as far as possible by packing control and use of the highest process compatible with quality, the canner could place the pack in cool warehouse storage. Progress of autosterilization of the spoilage organisms can then be followed through incubation tests at intervals.

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THE BACTERIOLOGY OF REFRIGERATED GROUND BEEF[a]

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The bacterial content of ground beef or hamburger received considerable attention during the early part of the twentieth century but little in recent years. Most of the early work was concerned with the numbers of bacteria in market hamburger and some of the authors suggested sanitary standards based on bacteria counts *(6, 13, 15, 21, 22)*. These standards have not received wide acceptance, however. Later work has considered kinds as well as numbers of bacteria in hamburger at the time of purchase *(4, 8)* and at the time of spoilage *(11, 19, 20)*.

It might be expected that modern emphasis on food inspection and sanitation would cause a marked improvement in the bacteriological quality of market hamburger as compared with the product available to consumers 30 or 40 years ago. In addition, the widespread use of mechanical refrigeration both in meat markets and in homes would be expected to reduce spoilage losses. Therefore, the study reported here was conducted to ascertain the bacteriological quality of hamburger purchased in a rather limited area and to determine the kinds of organisms that grew in this product and caused its spoilage during storage in an ordinary household electric refrigerator for several days. No report of a careful study of these organisms has been found in the literature.

EXPERIMENTAL METHODS

Plate counts. The meat was purchased directly from the showcase in the market, taken to the laboratory at once, and packed into an aluminum or glass container fitted loosely with a cover. This was kept in the meat-keeper tray of a household electric refrigerator immediately beneath the freezing unit where the temperature at the center of the meat was between 0° and 2°C.

Samples were removed at the time of storage and at 2-day intervals thereafter. Each consisted of a plug taken from the top to the bottom of the meat with a sterile ¾-inch cork borer. This sample was mixed thoroughly with a sterile spatula, then a 10-g. portion was blended for 3 minutes with 90 ml. of sterile water in a Waring Blendor. Appropriate dilutions were plated in duplicate in nutrient agar and incubated at 30°C. for 3 days. These conditions were selected after preliminary experiments had shown counts in nutrient agar to be as high or higher than those in several other media tried, and counts at 30°C. were practically identical to those at 20°C. The latter temperature is commonly used in studies of refrigerated foods but for most organisms it necessitates a longer incubation time.

Isolation and characterization of the predominant organisms. One of the plates used for the colony count of each sample was selected at random and a sector containing 50 (or in one experiment 25) isolated colonies was delineated with a wax pencil. Each of the colonies in the sector was picked into a tube of tryptone broth and incubated at 30°C. These cultures were used for subsequent tests and observations.

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[a] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[b] Deceased.

[c] Current addresses respectively: Route 3, Utica, Mississippi, and Pitman Moore Co., Indianapolis 6, Indiana.

Conventional descriptive tests *(5)* were run on the cultures. Unless otherwise indicated, incubation was at 30°C. Gram stains were made by the Hucker modification. Flagella were stained by Bailey's method as modified by Fisher and Conn. Motility was observed in hanging drop preparations. Cultures that were negative repeatedly by this method were tested in semi-solid agar as suggested by Tittsler and Sandholzer. Nitrate reduction was determined after incubation for 1 week in nitrate broth. Gore's method was used to test for indole production in 1% tryptone broth. Fermentation of glucose and lactose was measured in broth containing brom cresol purple indicator, with incubation for 1 week. Gelatin liquefaction was determined by Smith's modification of Frazier's method. Action in litmus milk was observed at intervals up to 30 days.

In addition, urease production was determined by the method of Stuart, Van Stratum, and Rustigian *(18)*. Sullivan's synthetic "K" medium as modified by Jamieson *(12)* was used to test for pigment production. Catalase formation was detected by adding about 2 ml. of 3% hydrogen peroxide to an agar slant culture. Growth at 37°C. was determined by observing broth cultures for visible turbidity at 1 week.

RESULTS

Plate counts in market hamburger. Aerobic plate counts for 20 samples of hamburger at the time of purchase are shown in Table 1. In 1914 Weinzirl and Newton *(22)* obtained counts ranging from 270,000 to 89,000,000 per g. for 44 samples of market hamburger. Forty of the counts were over 1,000,000 and 22 were over 10,000,000 per g. About 20 years later Geer, Murray, and Smith *(9)* obtained plate counts ranging from 8,000,000 to 44,000,000 per g. Table 1 shows that the hamburger used in this study was little if any better than the above in bacteriological quality. Two of the 13 samples from market D exceeded the standard of 10,000,000 per g. suggested by Weinzirl and Newton *(22)* and 7 out of the total of 20 samples from 6 markets failed to meet this standard.

Changes in hamburger during storage at 0° to 2°C. Samples 9 to 20 (Table 1) were stored for approximately 2 weeks and examined every

TABLE 1

Aerobic plate counts of hamburger at the time of purchase

Sample No.	Market	Plate count per g.[d]
1	E	4,700,000
2	C	11,000,000
3	B	95,000,000
4	A	34,000,000
5	A	12,000,000
6	A	7,300,000
7	D	29,000,000
8	D	16,000,000
9	D	4,900,000
10	D	3,200,000
11	D	6,400,000
12	D	5,200,000
13	D	3,600,000
14	D	1,800,000
15	D	1,400,000
16	D	2,300,000
17	F	45,000,000
18	D	8,900,000
19	D	7,100,000
20	D	2,800,000

[d] Samples plated in nutrient agar and incubated at 30°C. for 3 days.

other day. In most samples bacterial growth was very rapid and counts of 500,000,000 or more per g. were obtained within 6 days. Maximum counts for all samples were between 500,000,000 and 10,000,000,000 per g.

The most evident sign of spoilage of the hamburger was an undesirable odor. This can best be described as a stale, sour smell such as that usually associated with meat no longer fresh. Only in Sample 15 was there a putrid odor, which became evident near the end of the storage period. The time at which the characteristic sour odor was detectable naturally varied with different samples but usually it appeared between the 8th and 12 days. Fading of the color at the surface of the meat where it was exposed to air usually was noticeable at 4 to 6 days.

When the plate counts were plotted against time, 2 types of curves were obtained. Three of the samples showed the essential features of an ordinary bacterial growth curve, as is illustrated in Figure 1-A. The curves for the other samples, however, exhibited a rapid increase, then a sharp drop in numbers followed by a second increase to or near the initial peak. The time and extent of the drop varied with different samples but it was always significant. Because of the similarity between some of the curves, the results for certain of the samples were averaged and are shown in Figure 1-B and C.

The shapes of the curves which showed the drop in count followed by recovery are not particularly surprising when it is considered that the meat was contaminated with a variety of organisms with varying ability to grow at the temperature of storage. Thus it might be expected that one organism or group of organisms would grow rapidly at first, reach a limiting population and then die off. At the same time other organisms which grew more slowly or were present initially in lower concentration would eventually reach considerable numbers and thus account for the second rise in counts.

Kinds of organisms in hamburger during storage. Preliminary trials showed that gram negative, nonspore forming rods were predominant throughout the storage period (Samples 12-14). For Samples 15-18, therefore, 50 cultures were isolated at each sampling time and examined by tests that would indicate the genus of each isolate. By this means it was expected that a change in kinds of organisms would be demonstrated to coincide with the drop and subsequent rise in counts shown in Figure 1-B and C.

A few of the isolates from Samples 15-18 before storage were micrococci, but soon they were outnumbered by other organisms. The great majority of the isolates were gram negative, nonspore forming rods that produced acid but no gas from glucose, failed to ferment lactose, and did not produce urease. They grew well aerobically on agar but produced no visible pigment. Therefore, they were considered to be nonpigmented *Pseudomonas* and/or *Achromobacter* species. The greatest number of this group of cultures caused no visible change in litmus milk in 2 weeks. A smaller number produced an alkaline reaction.

Organisms with the characteristics of *Lactobacillus* species were isolated in varying numbers from all 4 samples. These were gram positive, nonspore forming rods that grew poorly on agar, did not produce catalase,

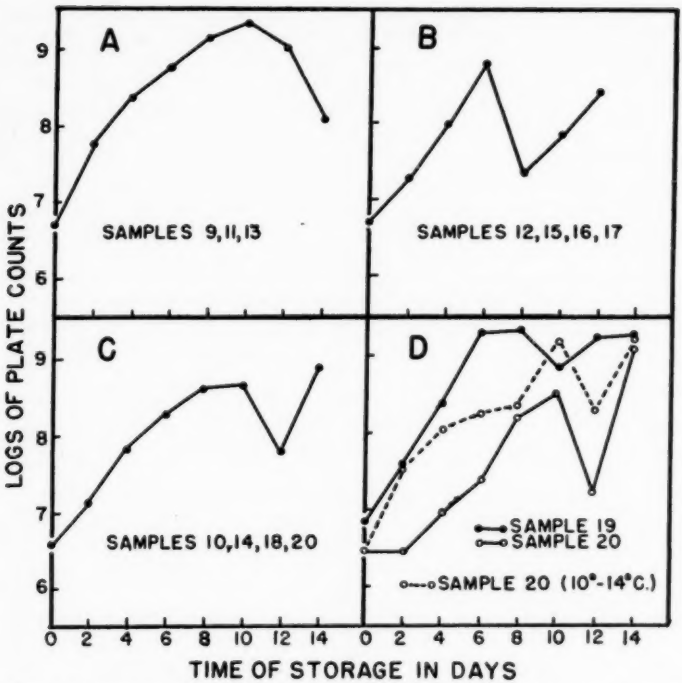

Figure 1. Plate counts of market hamburger during refrigerated storage. (Temperature 0°-2°C. except as indicated. A, B and C are averages.)

formed acid but no visible gas from glucose and lactose, and caused no change in litmus milk. In Sample 15 these organisms comprised almost half of the cultures from the initial plating and they increased in numbers rapidly, but after about 6 days they were overgrown by the gram negative rods. In Sample 18 the lactic organisms increased steadily throughout storage so that at 15 days they comprised 35% of the isolates.

Gram negative rods that produced urease were found in considerable numbers near the end of storage of Sample 15 but in no others. The detection of these organisms coincided with the development of a putrid odor in the meat. They were presumed to belong in the genus *Proteus*.

From the above statements it is clear that the predominant organisms in all 4 samples were nonpigmented *Pseudomonas* and/or *Achromobacter* species. Contrary to expectations, however, the tests used showed no clear-cut differences between the organisms that grew early in the storage period and those that grew later.

Two other samples of meat were stored as usual and the isolates were studied in greater detail in an effort to demonstrate a succession of organisms. In addition, part of one sample was stored in an ordinary ice box at

10° to 14°C. The plate counts are shown in Figure 1-D (Samples 19 and 20). Fifty cultures were isolated at each sampling period, but with Sample 20 these were divided equally between the lots at the two temperatures.

The main characteristics of the isolates are shown in Table 2. As usually is true in a study of this kind, it was impossible to identify the organism positively with species described in *Bergey's Manual (2)*. The

TABLE 2

The main differential characteristics of the organisms isolated from 2 samples of refrigerated hamburger

Characteristics	Group	No. of isolates	Genus*
I. Cocci, gram positive, miscellaneous	C	32	*Micrococcus, Sarcina*
II. Rods, nonspore forming, acid and no gas from glucose, indole negative, urease negative			
A. Gram positive, catalase negative, nonmotile, gelatin not liquefied, nitrates not reduced to nitrites, acid and no gas from lactose, no change or very slight acid in litmus milk	L	155	*Lactobacillus*
B. Gram negative, catalase positive, no acid or gas from lactose			
1. Green pigment produced, grow well at 37° C., motile with polar flagella, gelatin liquefied, nitrates reduced to nitrites, litmus milk rapidly peptonized becoming alkaline	P-1	9	*Pseudomonas (aeruginosa)*
2. No pigment produced, do not grow at 37°C.			
a. Motile with polar flagella			
(1) Gelatin liquefied			
(a) Litmus milk slowly peptonized, may be coagulated, reaction unchanged or slightly alkaline, nitrates reduced to nitrites	P-2	33	*Pseudomonas*
(b) Litmus milk partly reduced with soft coagulation, reaction unchanged or slightly alkaline, nitrates not reduced to nitrites	P-3	147	*Pseudomonas (geniculata)*
(c) Litmus milk reduced with firm coagulation, reaction becoming acid slowly, nitrates not reduced to nitrites	P-4	88	*Pseudomonas (fragi)*
(2) Gelatin not liquefied, nitrates not reduced to nitrites			
(a) Litmus milk partly reduced, reaction unchanged	P-5	202	*Pseudomonas*
(b) Litmus milk partly reduced, soft coagulation, acid reaction	P-6	62	*Pseudomonas (rugosa)*
b. Nonmotile, nitrates not reduced to nitrites			
(1) Gelatin liquefied, litmus milk partly reduced	A-1	9	*Achromobacter (butyri)*
(2) Gelatin not liquefied			
(a) Litmus milk partly reduced	A-2	11	*Achromobacter*
(b) Litmus milk partly reduced, soft coagulation, acid reaction	A-3	2	*Achromobacter (delmarvae)*

*The most closely related species in *Bergey's Manual (2)* is indicated in parentheses.

isolates were grouped, however, on the basis of the tests run and where a group differed only slightly from a described species, the probable species name is indicated in parentheses.

Except for the *Lactobacillus* species, most of which were found in Sample 19, the great majority of the isolates were motile, nonpigmented *Pseudomonas* species. These were about equally divided between gelatin liquefiers (Groups P-1 to P-4) and nonliquefiers (Groups P-5 and P-6). Only the 9 cultures in Group P-1 produced a visible pigment even when tested in the modified synthetic "K" medium recommended by Jamieson *(12)*. All of the P-1 organisms were isolated from a single portion of Sample 19.

The number of cultures in each group at each sampling period is shown in Table 3. It is evident that there was considerable difference between the flora of the two meat samples. In sample 19 the organisms responsible for the initial rapid increase in numbers (see Figure 1-D) were lactobacilli and motile gram negative rods that did not liquefy gelatin (P-5). As the count levelled off the proportion of gelatin liquefiers (P-1 to P-4) increased and remained fairly high throughout the storage period. The P-5 organisms showed a second increase that presumably accounted for the rise in count after the drop at 10 days.

In Sample 20, stored at the same temperature as No. 19, lactobacilli comprised only a small proportion of the isolates. Gelatin nonliquefiers (mainly P-6) grew first but later they were supplanted by the liquefying types (P-2 to P-4). This was essentially repeated in the portion stored in the ice box (10° to 14°C.) except that lactobacilli (Group L) grew rapidly for the first 4 days before they were outnumbered by the other organisms. The sharp rise in count in this sample between 8 and 10 days (see Figure 1-D) was caused almost entirely by increase in numbers of P-3 organisms.

The results in Table 3 fail to give the expected explanation for the increase in counts following the drop at 10 to 12 days. Rather than having only one or two kinds of organisms represented in the 14-day isolates, the flora was a generous mixture of many of the groups that had been found earlier in the storage period. The only evidence of a general change in the bacterial flora was a shift from a predominance of nonliquefying types to one in which a large proportion of the organisms were liquefiers of gelatin. This had no apparent relation, however, to the drop and subsequent rise of the counts.

DISCUSSION

It was a little surprising and somewhat disappointing to find that the bacteriological quality of the market hamburger used in this study was little if any better than that of the product available many years ago. There is no reason to think that the markets which supplied the samples were unusually unsanitary or that the food handlers were any more careless than those in other markets. As hamburger is usually handled in meat markets it is inevitable that the meat will become contaminated with organisms capable of growing rapidly at ordinary refrigerator temperatures. The extent of their growth and thus the time required for spoilage

TABLE 3

Group distribution of the isolates from two samples of refrigerated hamburger

Group[f]	Number of isolates in each group on the indicated days																							
	Sample 19								Sample 20															
	Stored at 0° to 2°C.								Stored at 0° to 2°C.								Stored at 10° to 14°C.							
	0	2	4	6	8	10	12	14	0	2	4	6	8	10	12	14	0	2	4	6	8	10	12	14
C (cocci)	8		1			1			3	8	6	1				1	3		3					
L (lactobacilli)	5	9	24	25	19	24	8	2		3		1	3	3	1	2		1	17	4			3	1
P-1				9																				
P-2	1				3					2	4		1	2		1		3	1	3	7			5
P-3	5				12	13	13	11				1	8	15	7	5		1			8	24	20	4
P-4					1	6		3		2	1	20	11	4	3	6		3		16	3	1		8
P-5	31	40	25	14	13	6	26	31	3	1					3		3	3	2		4			
P-6 (*Pseudomonas* sp.)					1		2	3	10	7	13		1			5	10	13	2		2			3
A-1												2			3	2								2
A-2									5	2			1		1	1	5							1
A-3 (*Achromobacter* sp.)									2								2							
Total isolates[h]	50	49	50	48	49	50	49	50	23	25	24	25	25	24	18[g]	23	23	24	25	23	24	25	23	24

[f] See descriptive key for the main characteristics of the groups.
[g] Only 20 colonies were picked rather than 25.
[h] The difference between the number of colonies picked and the number of isolates studied represents cultures lost for various reasons.

in the consumer's possession will depend mostly on the degree of contamination, the time the meat is held in the market, and, of course, the temperature at which it is stored. The success with which the ordinary meat handler controls the bacterial content of hamburger is suggested by the results in Table 1. Thus the purchaser receives a product already heavily inoculated and to prevent its rapid spoilage he must consume it promptly or freeze it.

The predominance of *Pseudomonas* species in the refrigerated hamburger is not in agreement with reports of other workers *(7, 10)* who found *Achromobacter* species predominant on chilled beef. This apparent discrepancy results from a change in nomenclature though, rather than an actual difference in the organisms. In the 6th edition of *Bergey's Manual (2)* the chief differential characteristic of the *Pseudomonas* genus is polar flagellation. In earlier editions this genus was reserved for organisms that produced a green pigment. Thus several polar flagellate, nonpigmented organisms formerly in the genus *Achromobacter* now are classified as *Pseudomonas* species.

The absence of *Flavobacterium* species was surprising because these organisms can grow at temperatures near 0°C. and have been reported in many studies of refrigerated foods, for example poultry *(14)*, herring *(1)*, and beef *(7)*. It must be assumed that the hamburger used in the storage trials simply was not contaminated to a significant extent with these organisms.

The frequent occurrence of lactobacilli in the hamburger samples also was unexpected. Lactic acid bacteria capable of growing at temperatures even a little below 0°C. were recognized years ago *(3)*, but one finds few reports of their occurrence in fresh meats. In fact, Empey and Scott *(7)* specifically state that they were unable to find lactobacilli in the surface flora of chilled beef. More recently, however, Niven *et al. (16)* described lactic organisms that caused greenish discolorations on the surfaces of cured sausages, and Steinke and Foster *(17)* isolated from refrigerated liver sausage large numbers of organisms having the characteristics of lactobacilli. Thus it would appear that lactic organisms are more common in meats than has been supposed.

The peculiar rise and fall in the plate counts shown in Figure 1 occurred too often to be an artifact. It was not unusual for a count to drop one log or more per g. (*e.g.* 500,000,000 to 50,000,000) in two days. The secondary rise in numbers resulted from growth of a mixture of organisms, all of which had grown earlier in the meat, rather than an increase by one or more kinds that had not been detected previously. We have no certain basis on which to explain this secondary increase in numbers. It might be conjectured that the meat underwent some kind of enzymatic change which made additional food material available to the organisms.

SUMMARY

Aerobic plate counts from 20 samples of market hamburger ranged from 1,400,000 to 95,000,000 per g. When samples of hamburger were stored in the meat-keeper tray of a household electric refrigerator at 0°

to 2°C., spoilage in the form of a sour odor first was noticeable at 8 to 12 days. By this time the counts had reached 500,000,000 or more per g.

The organisms in the meat when purchased were a mixture of gram negative, nonspore forming rods, various species of the family *Micrococcaceae*, and occasionally lactobacilli. The ones that grew most rapidly during storage at 0° to 2°C. were motile, nonpigmented members of the genus *Pseudomonas*. Five groups or species of this genus were found fairly consistently in the 2 samples of meat studied in detail. Of these, gelatin nonliquefying types were most numerous during the first few days of storage but liquefiers were found in large numbers after 6 to 8 days. A large majority of the total of 750 cultures isolated from the 2 samples of meat were unable to grow at 37°C.

Lactobacilli were isolated in varying numbers from all of 6 lots of hamburger during refrigerated storage.

Plate counts for 9 of 12 samples of hamburger held for about 2 weeks at 0° to 2°C. showed a sharp drop between the 6th and 11th day followed by a rise to or near the initial peak. This secondary increase was the result of renewed growth of some of the organisms that had multiplied earlier in the storage period.

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STUDIES ON TENDERNESS OF BEEF. I. RATE OF HEAT PENETRATION[a]

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The factors affecting the tenderness of beef are numerous, and include such diverse items as breeding, feeding and management, anatomy, cellular activity, enzymes, chemical changes, both analytical and physical, and cooking technics. Workers in a number of laboratories have been exploring various of these areas.

Beef generally is considered to be tender when first slaughtered, to become less tender with cold storage up to 48 hours, and then gradually to increase in tenderness as the storage period is lengthened. However, previous work by Paul, Lowe, and McClurg *(1)* indicated that beef cooked by roasting was least tender immediately after slaughter, and became increasingly tender with cold storage. Following the cooking method used by Ramsbottom, Strandine, and Koonz *(3)*, exploratory work by Paul, Bratzler, and Knight *(2)*, suggested that beef steaks cooked by frying in deep fat were more tender immediately after slaughter than after 24 to 48 hours of cold storage. It was postulated that the slow heating of beef in roasting hastened the development of rigor so that the roast became tough before being completely cooked, while the very rapid heating of the thinner steaks in deep fat frying prevented the development of rigor in the fresh samples. In order to test this theory, a series was designed to give direct comparisons of tenderness of roasts cooked in the oven with tenderness of steaks cooked in deep fat, after various periods of storage after killing.

PROCEDURE

The *semitendinosus* and *biceps femoris* muscles from 6 animals were utilized. Two of the carcasses were graded prime, 2 good, and 2 commercial. Each muscle was divided into 3 adjacent pairs of one-inch steaks and three- to four-inch roasts. The 6 pairs of steaks and roasts from each pair of muscles (right and left) were distributed at random over the various storage times of 0, 5, 12, 24, 48-53, and 144-149 hours. The time between stunning the animal and putting the meat on to cook or into cold storage was approximately one hour. After the appropriate period of storage at 4-7°C., each pair was cooked and sampled. The steaks were fried in deep fat at 147°C. to an internal temperature of 63°C. The roasts were cooked in an oven at 163°C. to the same internal temperature as the steaks. One-half inch cores were sheared on the Warner-Bratzler shearing machine for evaluation of tenderness. Records were kept of cooking times and losses. The pH of the raw meat was determined.

Samples for microscope examination were fixed in 10% formalin, sectioned on the freezing microtome, stained with Harris' haemotoxylin, and mounted in glycerine jelly.

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TABLE 1

Average cooking time, minutes per pound

	ROASTS						STEAKS					
Storage time, hrs.	0	5	12	24	48-53	144-149	0	5	12	24	48-53	144-149
Grade	*Semitendinosus*											
Prime	47.7	47.2	66.3	55.1	68.3	60.4	11.7	7.5	10.9	13.3	9.6	14.9
Good	45.5	65.4	69.8	73.9	81.1	66.6	10.4	10.4	15.8	14.4	13.4	15.1
Commercial	65.0	63.6	68.7	67.2	63.9	61.1	9.9	8.8	11.5	13.0	13.5	21.5
	Biceps Femoris											
Prime	21.8	33.8	39.3	39.8	38.1	37.4	12.8	9.5	13.8	9.7	6.6	12.9
Good	24.9	38.0	39.2	37.0	40.1	45.7	7.3	7.4	10.8	12.0	12.5	13.5
Commercial	32.1	39.6	43.8	39.0	42.8	34.5	8.1	11.5	12.3	10.4	15.2	10.3

TABLE 2

Average percent cooking losses

	ROASTS						STEAKS					
Storage time, hrs.	0	5	12	24	48-53	144-149	0	5	12	24	48-53	144-149
Grade	*Semitendinosus*											
Prime	12.1	13.4	16.8	14.5	14.0	17.7	19.6	21.8	25.5	28.2	20.4	24.3
Good	8.4	9.7	9.2	15.6	17.6	18.6	18.5	17.6	28.4	28.2	24.8	22.7
Commercial	13.7	13.7	13.6	14.4	13.6	16.0	19.3	20.7	24.0	24.8	24.7	30.3
	Biceps Femoris											
Prime	11.5	13.7	16.3	16.4	13.5	13.6	23.7	28.2	26.2	23.6	18.0	21.0
Good	11.0	13.4	12.3	12.6	18.1	11.9	17.4	20.7	25.8	32.3	26.5	19.5
Commercial	12.6	14.8	15.4	14.6	14.1	14.9	19.2	22.2	27.5	26.6	24.4	19.5

TABLE 3

Average force required to shear sample cores

		ROASTS						STEAKS					
Storage time, hrs.		0	5	12	24	48-53	144-149	0	5	12	24	48-53	144-149
Animal, grade		*Semitendinosus*											
1	good	23.14	18.93	22.39	19.29	19.25	13.46	12.00	13.29	15.93	22.39	20.57	12.61
2	good	19.04	16.04	18.71	17.25	17.14	13.57	12.57	18.32	21.64	17.93	15.25	10.07
3	prime	18.43	22.50	15.71	14.53	17.61	8.68	16.68	18.71	19.96	12.86	18.64	8.36
4	prime	15.04	19.75	15.14	9.86	20.07	12.21	7.18	18.29	19.54	21.57	17.07	12.93
5	commercial	23.73	20.89	16.68	16.46	15.96	16.46	14.18	15.89	19.29	18.61	13.18	16.68
6	commercial	20.32	21.57	24.25	16.61	15.61	13.04	10.96	17.61	18.21	19.96	18.11	9.29
		Biceps Femoris											
1	good	20.36	16.18	17.07	25.43	7.86	7.74	11.46	10.11	14.89	17.07	11.61	8.43
2	good	23.11	18.11	15.11	11.25	16.39	9.18	12.29	19.61	16.82	16.54	19.25	16.50
3	prime	10.36	13.75	13.36	9.18	6.50	6.29	8.71	13.68	17.18	11.93	8.04	9.11
4	prime	17.39	14.82	12.21	8.39	8.61	7.07	9.61	14.46	12.89	10.61	9.43	12.04
5	commercial	17.07	10.68	12.64	11.82	7.68	11.00	11.79	16.57	15.61	12.71	13.86	6.71
6	commercial	18.54	17.39	16.68	11.46	11.89	7.71	11.82	16.46	11.71	19.54	11.93	6.39

RESULTS

Cooking time. The minutes per pound required to raise the temperature at the center of each cut to 63°C. are listed in Table 1.

Within each cooking method, the cooking time required was approximately the same, when allowance was made for the fact that the cuts with no storage were at a higher internal temperature at the beginning of cooking than were the cuts which had been held under refrigeration. However, deep fat frying required much less time than roasting in the oven, reflecting the effects of thickness of cut and efficiency of heat transfer.

Cooking losses. The percent cooking losses are given in Table 2. The cooking losses tended to increase somewhat with cold storage up to 24 hours, then remained about constant. The steaks had considerably higher cooking losses than the roasts, but there was no consistent difference due to grade or muscle used.

It is of interest to note that within each cooking method, as the cooking time increased, the cooking losses usually increased. However, this did not hold between the methods of cooking, as deep-fat frying shortened the cooking time, but increased the cooking losses, in comparison with roasting.

pH readings. The pH of the raw meat decreased with storage, rapidly for the first 24 hours, more slowly thereafter. The values for the 6 animals were quite close, the averages being as follows: zero storage, pH 6.68; 5 hours, 6.50; 12 hours, 6.23; 24 hours, 5.90; 48-53 hours, 5.55; 144-149 hours, 5.48. These figures are in general agreement with those reported by others.

Shear. The average pounds shear force are listed in Table 3. The significant F-values from analysis of variance are given in Table 4.

TABLE 4

Significant F-values from analysis of variance of shear force data

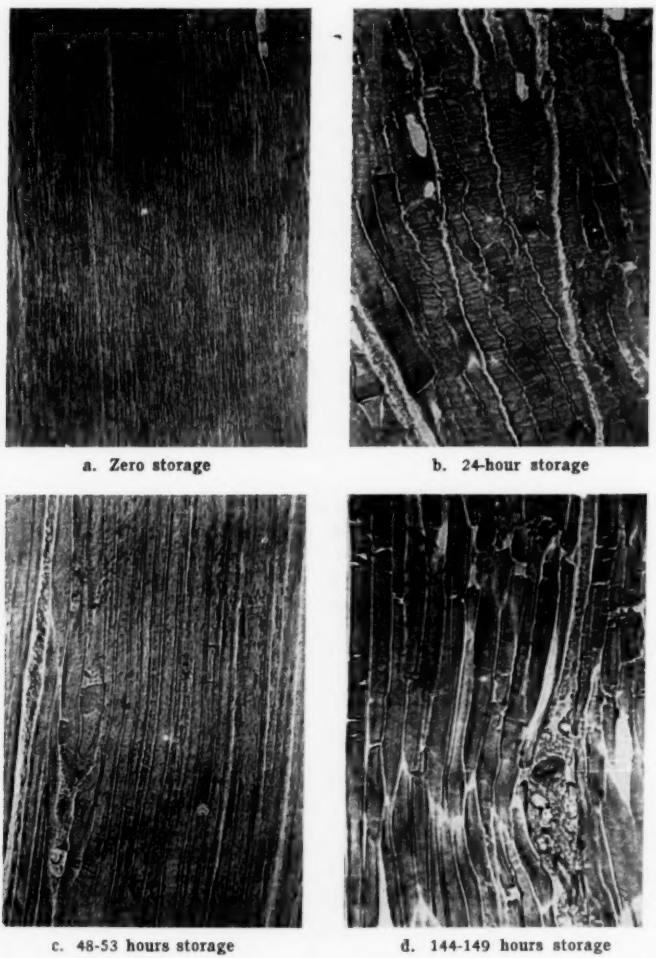
Source	F-value	
	Semitendinosus	*Biceps Femoris*
Storage time	10.18**	8.84**
Method of cooking	5.67*	
Grade		11.13**
Replication		5.08*
Storage by method of cooking	6.03**	6.41**

*$p<0.05$. **$p<0.01$.

Length of cold storage caused marked changes in tenderness as measured by shear force. However, the pattern of change differed with the method of cooking. The roasts were generally least tender with no storage, and increased in tenderness with increasing storage. The steaks decreased in tenderness with storage up to 24 hours, then returned to approximately their original tenderness after 144-149 hours of storage. The steaks from the *semitendinosus* muscle were more tender than the roasts. In the *biceps femoris*, the method of cooking did not result in a significant difference. The cuts from the *biceps femoris* showed a highly significant difference due to grade (prime most tender, good least tender), and a significant

difference due to replication (the first animal within each grade was more tender than the second).

Histological appearance. There is a fairly well defined pattern of changes which can be observed microscopically during the onset and dissolution of rigor in raw beef. In fresh beef, before rigor has developed, the fibers are straight, poorly differentiated, and the longitudinal striations are quite prominent (Figure 1, a). As the muscle goes into rigor, evidence



a. Zero storage b. 24-hour storage

c. 48-53 hours storage d. 144-149 hours storage

Figure 1. Histological appearance of raw semitendinosus after various periods of cold storage, magnification 45 ×.

of severe contraction appears in the formation of rigor nodes and Z-Z contractions (Figure 1, b). The Z-Z contraction has been attributed to a passive wrinkling of uncontracted fibers. However, the frequent occurrence of this form in sections where no rigor nodes are found suggests that this also may be an active response to forces produced by the development of rigor. As rigor diminishes, the fibers tend to relax and become more nearly straight. Cracks begin to appear (Figure 1, c). With longer

a. Roast, Zero storage

b. Roast, 144-149 hours storage

c. Steak, Zero storage

Figure 2. Histological appearance of cooked semitendinosus, magnification 45 ×.

storage, these cracks develop into definite breaks and granulated areas in the fibers (Figure 1, d). The active contraction in rigor is thought to contribute materially to the toughness of the meat, while the relaxation as rigor passes makes the meat more tender, and the breakdown of the muscle fibers increases this tenderizing effect.

The samples of meat cooked by roasting show the same pattern of development and dissolution of rigor as the raw samples except that the changes are accelerated. The roasts cooked after zero storage show the nodes and Z-Z contractions of active rigor (Figure 2, a) while those cooked after cold storage have a greater number of breaks and areas of granulation (Figure 2, b).

The steaks fried in deep fat after zero storage show evidence of heat rigor rather than the normal rigor picture. Heat rigor is evidenced by clumping of the muscle substance (Figure 2, c). Heat rigor apparently does not result in less tender steaks, as evidenced by the shear data. The steaks held in cold storage before cooking had about the same microscopic appearance as the raw samples.

SUMMARY

A comparison was made of steaks fried in deep fat with roasts cooked in the oven, after 0, 5, 12, 24, 48-53 and 144-149 hours of cold storage after slaughter. The *semitendinosus* and *biceps femoris* muscles from 6 animals were utilized. Two of the animals graded prime, 2 good, and 2 commercial.

The steaks required a much shorter time to cook, but had higher cooking losses than the roasts. However, within each method of cooking, the cooking losses generally increased with longer cooking time. The cooking losses tended to increase with storage through 24 hours, but were not affected appreciably by grade of animal or by muscle utilized.

The pH value of the raw meat decreased with increased cold storage.

The tenderness, as measured by force required to shear, changed with storage. The roasts were least tender immediately after slaughter, and became increasingly tender as the time of cold storage lengthened. The steaks were tender immediately after slaughter, became less tender with cold storage up to 24 hours, then returned to approximately their original tenderness with storage of 144-149 hours.

It is believed that the slow heat penetration during roasting induced the development of rigor in the roasts cooked immediately after slaughter, while the rapid heat penetration during deep fat frying coagulated the muscle substance before the zero-storage steaks could go into rigor. This is borne out by the histological sections, as those from the zero-storage roasts showed evidence of active rigor contractions, while those from the zero-storage steaks showed the presence of heat rigor instead.

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INFLUENCE OF LIGHT ON STABILITY OF REDUCED ASCORBIC ACID IN EXTRACTS OF VEGETABLES[a]

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(Manuscript received February 16, 1952)

The nutritive value of vegetables and fruits changes during storage, marketing, and processing, but the factors introduced in handling do not exert the same destructive effect on different products. There is indication that the rate and degree of change are governed by an inherent property of the specific products. Reder *(5)* has demonstrated that the presence of extracts of vegetables alter the rate of oxidation of an ascorbic acid solution, the alteration being an inhibition which varies with the vegetable.

This investigation is an explanation of the factors inherent in vegetables which effect a maintenance of ascorbic acid content. It is limited to the water-soluble factors and is not a study of the system of the intact product. Extracts of 6 vegetables are compared as to their relative abilities to retain ascorbic acid at their natural pH, and the retention of each in the dark and on exposure to light is compared.

The study of the effect of light on these systems was prompted by the work of Kon *(4)* who made the claim that the short waves of visible light were responsible for the oxidation of ascorbic acid in milk. Holmes and Jones *(3)* reported the simultaneous loss of riboflavin and ascorbic acid in milk on exposure to light. Dodds, MacLeod, and Carr *(2)* demonstrated the photosensitized oxidation of reduced ascorbic acid in blood serum and urine and noted acceleration of the reaction by increasing riboflavin content.

EXPERIMENTAL PROCEDURE

Extracts of sweet potato, Irish potato, lettuce, green beans, asparagus, and cabbage were investigated. The natural pH of the vegetables was found by macerating with distilled water in a Waring blendor, filtering the puree and determining the pH of the filtrate.

The samples were made by blendor extraction of the vegetables with 1% oxalic acid. This was filtered, then returned to the predetermined pH of the vegetable by the addition of MacIlvane's citrate-phosphate buffers. The final volume was adjusted to a 1 to 7 dilution of the vegetable. This was sample A. This constant proportionality between weight of vegetable and volume of extractant allowed a comparison between vegetables even though it furnished samples of unequal ascorbic acid content.

Sample B was constituted so that the ascorbic acid content was equal to that of Sample A, with one-half the ascorbic acid provided by the vegetable extract (Sample A), the other half by a solution of synthetic ascorbic acid at the required pH. Thus in this series, B, the vegetable content was one-half the amount in Series A.

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[b]From a thesis presented in partial fulfillment of the requirements for the Master of Science degree.

Fifty-ml. samples of A and B in stoppered 300-ml. pyrex Erlenmeyer flasks were stored in the dark and exposed to light, keeping the temperature the same for both conditions. The light was furnished by 2 parallel 30-watt fluorescent daylight tubes. The intensity was 490 ± 10 foot candles. The extracts were analyzed for reduced ascorbic acid immediately after preparation and at various time intervals of exposure. These intervals were determined by overall time for oxidation, and varied from 15 minutes to 2 hours. An effort was made to have 5 to 8 analyses for each sample to establish rate of oxidation.

Solutions of synthetic ascorbic acid buffered to the pH of the vegetable extracts and in the same concentration range served as blanks. They were exposed to light and dark as were the vegetable extracts.

OBSERVATIONS AND DISCUSSION

The data presented in Figures 1 to 7 inclusive are percentage retentions based on the original concentration of ascorbic acid. The original concentration varied because the experimental conditions were set up to provide equivalent extractions of equal weights of the raw vegetables. Table 1 shows initial ascorbic acid content for one series. These values are typical. The data presented in Figures 1 to 6 are from duplicate analyses of two typical runs, each curve is derived from the two runs indicated.

TABLE 1

Typical initial ascorbic acid content of vegetables and extracts

Vegetables	Vegetable	Extract	Extract alone	Extract and ascorbic acid
	mg. a.a./ 100 g.	*pH*	*mg. a.a./ 100 ml.*	*mg. a.a./ 100 ml.*
Sweet potato	25.04	6.0	3.13	2.87
Asparagus	32.96	6.2	4.12	4.15
Irish potato	10.64	6.2	1.33	1.57
Cabbage	54.88	6.4	3.43[c]	3.29[c]
Green beans	20.08	6.6	2.51	2.35
Lettuce	12.40	6.6	1.55	1.26

[c] Further 1 : 1 dilution.

Sweet potato. Data on the retention of ascorbic acid in sweet potato extract at pH 6.0 are shown in Figure 1. In solution A the destruction of ascorbic acid in the extract kept in the dark was more rapid than that of the control. When exposed to light the oxidation was accelerated still more and the reduced ascorbic acid disappeared in 7 hours. In solution B, which had an equivalent concentration of ascorbic acid but one-half as much vegetable as A, the oxidation was slower than A in both light and dark. The light exposure accelerated the oxidation in both solutions.

Asparagus. Retention of ascorbic acid in asparagus extract at pH 6.2 is shown in Figure 2. For both solutions A and B the rate of oxidation in the dark was approximately the same as for the blanks, while the destruction on exposure to light was complete in 3 hours.

Irish potato. Retention of ascorbic acid in Irish potato extract, pH 6.2, is given in Figure 3. Oxidation of ascorbic acid in the extracts in the dark was more rapid than that of the blank; on exposure to light all of the ascorbic acid was oxidized in 4½ hours.

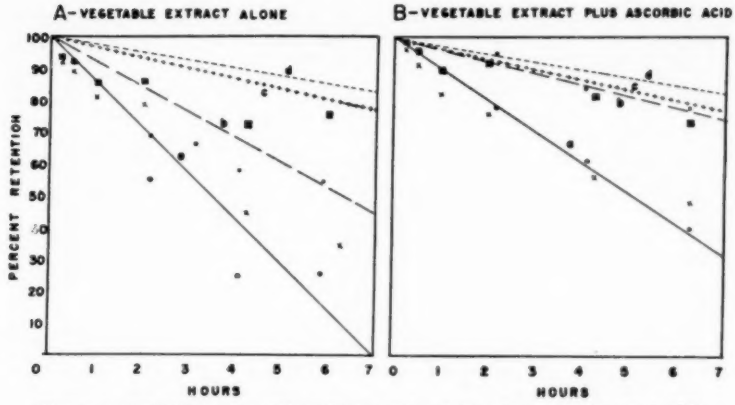

Figure 1. Retention of ascorbic acid in sweet potato extract, pH 6.0, 28 ± 1°C.
a. In light, 490 ± 10 foot candles, × run No. 1, ○ run No. 2.
b. In the dark, ⊠ run No. 1, ● run No. 2.
c. Ascorbic acid solution, pH 6.0, in light, 490 ± 10 foot candles.
d. Ascorbic acid solution, pH 6.0, in the dark.

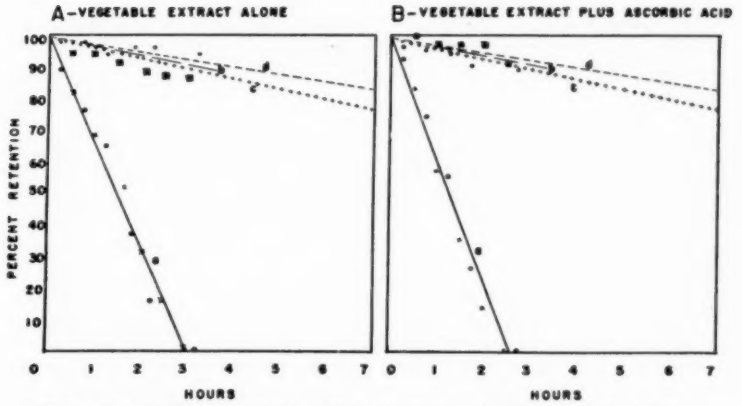

Figure 2. Retention of ascorbic acid in asparagus extract, pH 6.2, 28 ± 1°C.
a. In light, 490 ± 10 foot candles, × run No. 1, ○ run No. 2.
b. In the dark, ⊠ run No. 1, ● run No. 2.
c. Ascorbic acid solution, pH 6.0, in light, 490 ± 10 foot candles.
d. Ascorbic acid solution, pH 6.0, in the dark.

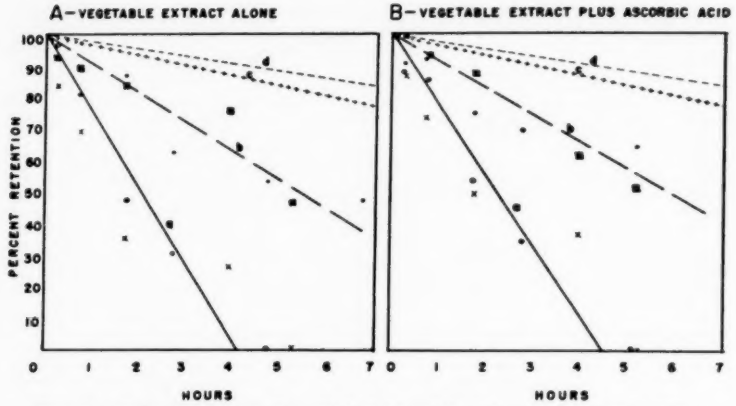

Figure 3. Retention of ascorbic acid in Irish potato extract, pH 6.2, 28 ± 1°C.
a. In light, 490 ± 10 foot candles, × run No. 1, ○ run No. 2.
b. In the dark, ☒ run No. 1, ● run No. 2.
c. Ascorbic acid solution, pH 6.0, in light, 490 ± 10 foot candles.
d. Ascorbic acid solution, pH 6.0, in the dark.

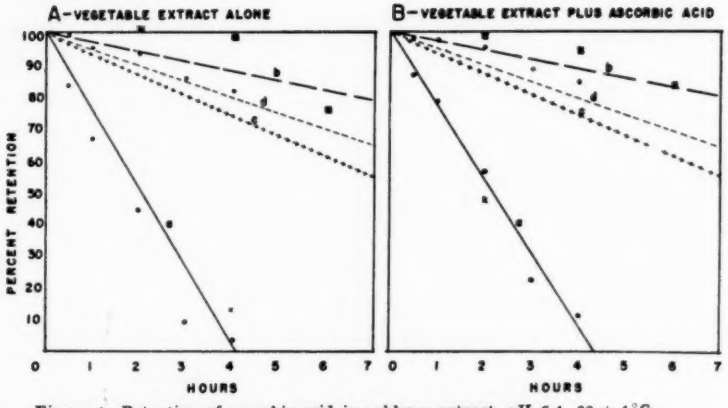

Figure 4. Retention of ascorbic acid in cabbage extract, pH 6.4, 28 ± 1°C.
a. In light, 490 ± 10 foot candles, × run No. 1, ○ run No. 2.
b. In the dark, ☒ run No. 1, ● run No. 2.
c. Ascorbic acid solution, pH 6.6, in light, 490 ± 10 foot candles.
d. Ascorbic acid solution, pH 6.6, in the dark.

Cabbage. Data on retention of ascorbic acid in cabbage extract, pH 6.4, are furnished in Figure 4. An inhibition of the oxidation was shown by the extract in the dark; the rate was slower than that of the blank. There was an acceleration of oxidation in the light, all the ascorbic acid being oxidized in 4½ hours.

Green Bean. Retention of ascorbic acid in green bean extract, pH 6.6, is shown in Figure 5. An inhibition of oxidation was shown by the extract kept in the dark; the rate of loss was slower than that of the blank. The light sensitized loss was accelerated but the shape of the curve indicates interference by the inhibiting factor after approximately 2 hours of exposure. The inhibition is lessened at half dilution (B) so that oxidation was completed in 7 hours in the light.

The presence of an ascorbic acid oxidation inhibitor in snap beans along with indications of its properties has been reported by Branthoover and Hard *(1)*.

Lettuce. Retention of ascorbic acid in lettuce extract, pH 6.6, is shown in Figure 6. The loss of ascorbic acid in the dark was faster than that in either blank, while the acceleration with light exposure was very marked, the loss being complete in less than 3 hours.

Figure 7 presents curves for the samples of Series A shown in Figures 1 to 6, permitting direct comparison of the oxidation of the ascorbic acid of these vegetable extracts both in the dark and in the light. The relative rates of destruction of ascorbic acid in the extracts kept in the dark were: lettuce and Irish potato about equal and the most rapid, then sweet potato, asparagus, ascorbic acid blanks, cabbage, and green beans the slowest. The relative rates of destruction of the ascorbic acid in the extracts exposed to light were: lettuce, again most rapid but almost equalled by asparagus, then Irish potato and cabbage also about equal, followed by sweet potato, and green beans, again the slowest.

Reder *(5)* demonstrated the inhibiting action of vegetable extracts on ascorbic acid solutions. Boiled extracts were added in amounts equivalent to 0.5 to 1 g. of fresh vegetable to unbuffered solutions of ascorbic acid, 1 mg. in 40 ml., and boiled. For the 5 vegetables common to both studies, the order of increasing effectiveness of inhibition is given as lettuce, sweet potato, cabbage, Irish potato, and green beans. While the order, except for the extremes—lettuce and green beans—is not identical with that reported here, neither were the conditions employed by Reder *(5)* identical with those used in this investigation.

SUMMARY AND CONCLUSIONS

Oxalic acid extracts of sweet potatoes, Irish potatoes, lettuce, green beans, asparagus, and cabbage were prepared, and each extract was buffered to the natural pH of the specific vegetable which it represented. Samples of the buffered extracts, at each of 2 dilutions, were exposed to light of a standard intensity; identical samples were kept in the dark. At various time intervals the samples were analyzed for reduced ascorbic acid.

The oxidation of ascorbic acid in all of the extracts studied was greatly accelerated when the extracts were exposed to light.

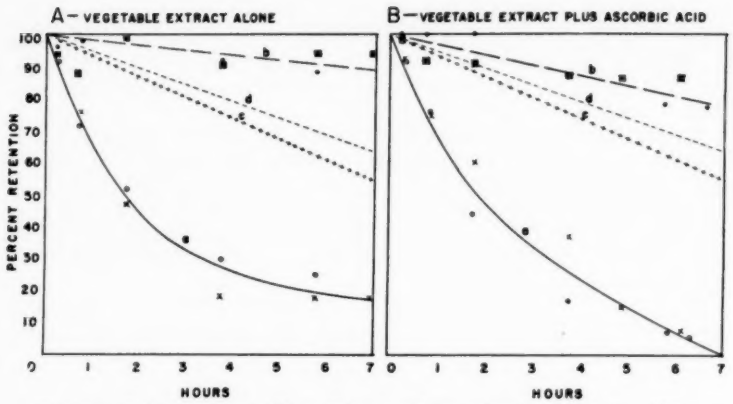

Figure 5. Retention of ascorbic acid in green bean extract, pH 6.6, 28 ± 1°C.
a. In light, 490 ± 10 foot candles, × run No. 1, ○ run No. 2.
b. In the dark, ⊠ run No. 1, ● run No. 2.
c. Ascorbic acid solution, pH 6.6, in light, 490 ± 10 foot candles.
d. Ascorbic acid solution, pH 6.6, in the dark.

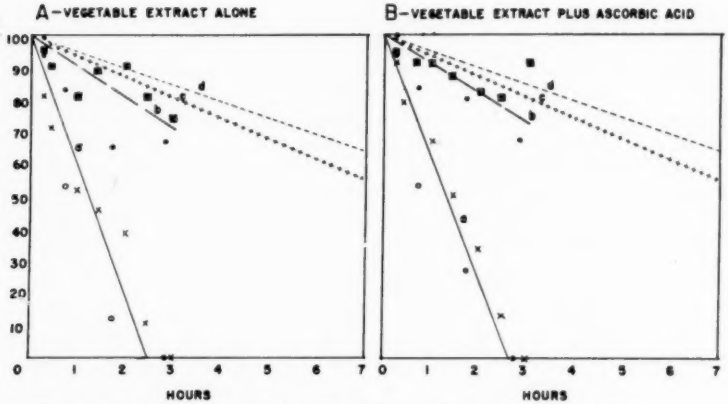

Figure 6. Retention of ascorbic acid in lettuce extract, pH 6.6, 28 ± 1°C.
a. In light, 490 ± 10 foot candles, × run No. 1, ○ run No. 2.
b. In the dark, ⊠ run No. 1, ● run No. 2.
c. Ascorbic acid solution, pH 6.6, in light, 490 ± 10 foot candles.
d. Ascorbic acid solution, pH 6.6, in the dark.

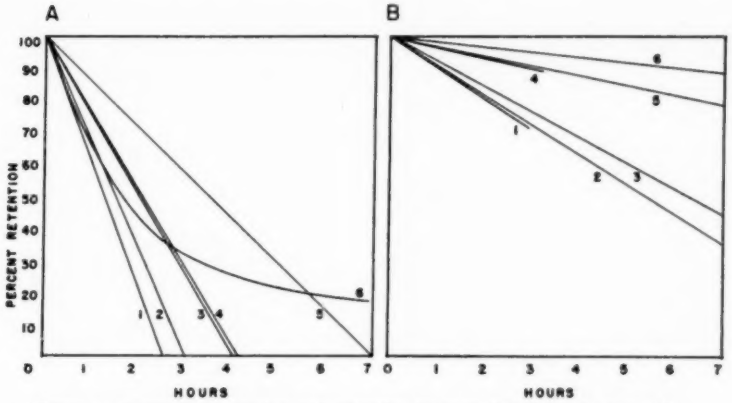

Figure 7. Summary of ascorbic acid retention by vegetable extracts, 28 ± 1°C.

A. In light, 490 ± 10 foot candles
1. lettuce
2. asparagus
3. Irish potato
4. cabbage
5. sweet potato
6. green bean

B. In the dark
1. lettuce
2. Irish potato
3. sweet potato
4. asparagus
5. cabbage
6. green bean

The Irish potato and lettuce extracts accelerated the rate of oxidation of ascorbic acid even in the dark.

Green bean and cabbage extracts inhibited the oxidation of their ascorbic acid when the extracts were protected from light.

The rate of destruction of ascorbic acid in the vegetable extracts was the same at the original concentration and at one-half strength except in the sweet potato and perhaps the cabbage extracts.

Rate of oxidation of ascorbic acid in the green bean extract decreased significantly after a short period of rapid oxidation of the beginning of the exposure.

The amount of destruction of ascorbic acid in the six vegetable extracts was shown to vary with different vegetables and with different dilutions of the same vegetable extract.

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HEAT PROCESSING OF BEEF

II. DEVELOPMENT OF ISOTHERMAL AND ISOCHRONAL DISTRIBUTIONS DURING HEAT PROCESSING OF BEEF[a,b]

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(Manuscript received April 12, 1952)

Ball (*2,3*), Bigelow (*4*), Schultz and Olson (*9*), and others (*7,8,11*) have developed graphical and mathematical methods for the determination of the length of thermal processes necessary to sterilize a great variety of foodstuffs sealed in cans. These investigations necessitated the study of both the mechanism of heat transmission into the can and the behavior of bacteria subjected to the heat treatment.

These investigators emphasized the study of time-temperature relationships at the geometrical center of the container as the point of slowest heating. The formulae and methods developed pertained to the same location where, it was contended, the probability of the survival of bacteria was the highest.

In later investigations Stumbo (*10*) drew the attention of future experimenters to the importance of temperature distribution in time and in space as important factors in the study of heat process evaluation. He analyzed the methods of process evaluation and found that the mechanism of heat transfer within the container should be considered for greater accuracy in process determination. He also showed in this analysis that consideration of the lethality of heat treatment in the center of the container as the slowest heating point may not always be the true criterion of the sterilization capacity of a thermal process. It was pointed out that locating the position, not necessarily in the geometrical center of the container, where the probability of bacterial survival is the greatest would be of paramount value in process evaluation.

Awberry and Griffith (*1*) determined the specific heat of fresh beef in the range from 18° to 48°C. as 0.62 cal. per g. per °C. This value checks closely with the value for specific heat found by Macintire (*6*) as 0.77. The value of thermal conductivity was found to be 0.00389 cgs units; density at room temperature 1.169 g. per cc. Thermal diffusivity (thermal conductivity/specific heat x density) was found to be 0.0090 sq.cm. per second. In order to check the results obtained a comparative method was used to avoid certain boundary conditions imposed by the theory. All of the determinations made by Awberry and Griffith were made on meat in the form of slabs using the hot-plate method. The importance attached to the thermal diffusivity con-

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[b] This paper reports research sponsored by the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned No. *383* in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

stant was explained by the authors by the fact that "It is the quantity which governs the rate of approach to a steady régime when thermal conditions are altered." Several other workers have contributed to the study of thermal properties of meat in hermetically sealed containers, however, none of these investigations dealt directly with the temperature distributions in the container undergoing thermal treatment.

Physical, chemical, and bacteriological changes in meat during processing progress to different extents at the various locations in the container at any given time. In processes where a considerable temperature gradient is maintained substantially throughout the process, whatever the cause, large differences in the effects of processing may exist among locations in the container. If a heat process is to be evaluated efficiently in terms of changes in tenderness, palatability, color, consistency, etc. of the product or in terms of its sterilizing effect these changes must be considered in the light of their distribution in the container during processing.

EXPERIMENTAL PROCEDURE

The present investigation was undertaken in an effort to describe the temperature changes in solid pieces of beef in cans as a function of processing time and location of the measured points in the meat.

No. 2 cans sealed under 25 inches of vacuum were processed in steam in a vertical, cylindrical type processing retort. The temperatures were measured and recorded by an automatic multipoint self-balancing potentiometer recorder. A box with shorting out switches was added to the instrument to facilitate the reading of individual thermocouples. The thermocouples used were copper-constantan molded in thermosetting plastic. The length varied from 1¾ inches to 3⅜ inches.

Calibration of the temperature measuring devices. The procedure adopted for determining the accuracy of temperature measurements was to calibrate the temperature measuring devices as a unit, i.e., thermocouples, thermocouple extension wires and the recording potentiometer. As the actual determinations in the test fell within the range of 50 to 270 deg. F., the melting points of ice, naphthalene, benzoic acid and salicylic acid of analytical grade, were chosen to give four points in the range of temperatures from 32 to 319.64 deg. F. The method of calibration used in this experiment was that of a melting point determination. This procedure was chosen because such determinations for the substances used are not significantly affected by the changes in atmospheric pressure.

Two glass capillary tubes were attached to the tested thermocouple which was immersed in a castor oil bath stirred by an electric mixer to assure even temperature distribution within the bath. The rate of heating was maintained low in the vicinity of the melting point. The beginning and the end of melting was recorded on the potentiometer chart for each of the two capillary tubes. Four runs were made for each thermocouple to determine the actual melting point. During each run a second thermocouple was tested to check the results. Both thermocouples were located as close together as possible. Eight thermocouples were calibrated, two from each of four sets of eight thermocouples each, chosen at random.

The deviations from the true temperature line were computed and the average of the deviations from the true temperature line were found to be:

32.00°F.	−0.80°F.
177.44°F.	−0.23°F.
252.50°F.	+0.30°F.
319.64°F.	−2.07°F.

The observed melting points fell fairly close to the true temperature line and on both sides of it. The only large deviation occurred at the temperature of 319.64°F. This could have been due to the difficulty in observing the melting which occurred in a relatively short period of time. The deviations in the range of temperatures from 32–252°F. which was essentially the range used in the investigation were less than the precision in reading the temperatures permitted by the potentiometer charts (±0.5°F.). In view of these results the observed errors were neglected.

Another possible source of error lies in the expansion of the potentiometer charts due to the humidity changes in the surrounding air. Observations indicated that maximum expansion at the extreme end of the chart was equivalent to 0.5°F. and in the center 0.25°F. As the temperature differences were used in calculations, the errors due to the chart expansion may also be considered negligible.

Meat processing. Of the factors influencing changes in meat during processing, time, temperature, and location of the measured points in the container were considered of primary importance in this investigation. The effect of processing time, an important variable in heat process evaluation was minimized by allowing the duration of the test run to be such that the slowest heating point in each can ultimately reached a temperature 3–4°F. below the processing temperature.

Because of the fibrous construction of meat, a cylindrically shaped piece of meat was placed with the fiber direction parallel to the central vertical axis of the can. A single solid piece of meat was used to avoid excessive influence of convection. The research was limited to a single cut from the animal, the beef shoulder clod (canner and cutter grade).

As no data were available indicating a possible scheme for the test procedure and the number of variables was considerable it was found desirable to make a set of preliminary tests with the purpose of establishing the trend of functions under consideration and to aid in designing the final test. The experimental techniques were the same throughout the preliminary and final tests.

In the preparation of the meat the clod was trimmed of excessive fat, skin and connective tissue, and cylindrically shaped pieces of approximately the dimensions of a No. 2 can were cut from the clod, packed in cans and sealed.

A five minute venting period was allowed prior to processing to remove air from the retort. The steam was shut off when the slowest heating point being measured reached a temperature 3–4°F. below the retort temperature and the cans were cooled in water.

Preliminary investigations indicated that the heating curves follow fairly well the theoretical relationship (Eq. 33, part I) in its first approximation, which is:

$$CT = RT - (RT - IT)A_{11}e^{-k(\mu_1^2+\lambda_1^2)t}J_0(\mu_1 r)\sin\lambda_1(z+l) \qquad \text{Eq. 1}$$

for a No. 2 can where the meaning of the symbols is as in Eq. 33, Part I and the values of the constants for a No. 2 can are:

λ_1	0.275
μ_1	0.572
a	4.35 cm
l	5.80 cm
A_{11}	2.04

The presence of two, three or four thermocouples in one can did not disturb the heat flow pattern and the use of four thermocouples offered the advantage of an entire replication of the complete set of points being tested within a single clod.

The final test, therefore, was made by randomizing the influences of positions (Figure 1), clods and temperatures using randomized Latin Squares.

Thirty-two runs were made which resulted in four replications of each point through two temperature ranges.

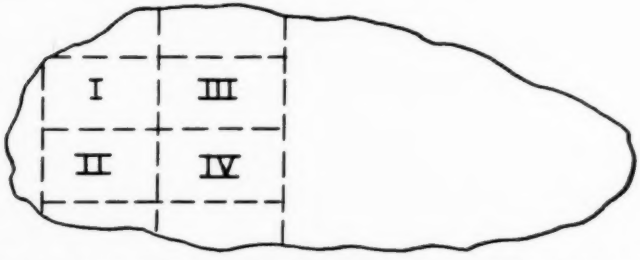

Figure 1. Top view of the beef shoulder clod showing the location of positions in the cut.

Twelve locations were tested which were distributed in the central part of the container not less than ¾ inch from the top or the bottom of the can, and not less than ½ inch from the side walls. The distribution of the points (Figure 2) was symmetrical to facilitate interpolation of the isothermals on a diagram.

During the final test runs, two retort temperatures (225° and 255°F.) were used. Each can contained one solid piece of lean canner and cutter grade, beef shoulder clod with fibers parallel to the central axis of the can. The points at which the temperatures were measured were distributed as shown on Figure 2. Approximately 600 g. of meat at a uniform temperature of 60°F. was filled into each can, vacuum sealed, processed and cooled in water.

Data were evaluated for the twelve locations indicated in Figure 2 at two processing temperatures (225° and 255°F.) for each location, four heating curves were determined (Figures 3 and 4), which represented the four replicates.

Heating curves, recorded on the potentiometer charts, were transcribed at 10-minute intervals. The starting point was the time when the steam was turned on, and the initial

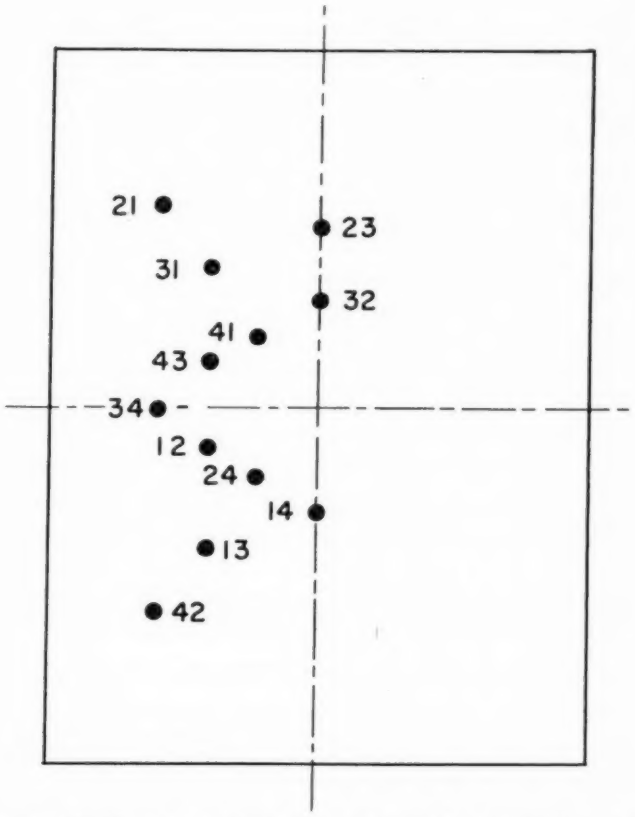

Figure 2. Distribution of locations tested during the final investigation. Vertical cross-section through central axis of No. 2 can.

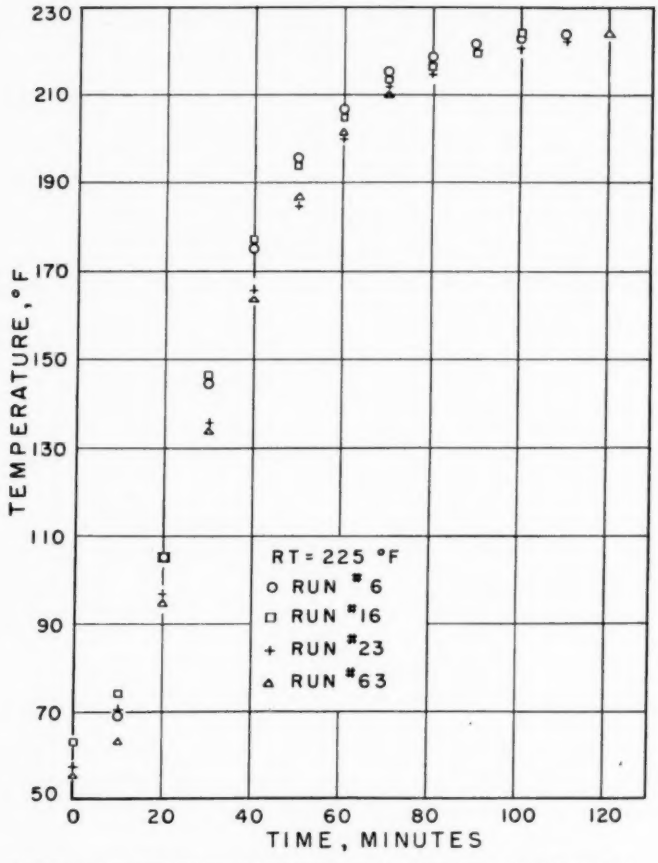

Figure 3. Four replications of heating curves for Location 23 (RT is 225° F.).

temperature differences were registered at this time. However, computations of the errors and corrections required were made with the ten minute readings considered as the first observation taking into account the initial temperature time lag.

RESULTS AND CALCULATIONS

For the purposes of the investigation it was assumed that the isothermal surfaces were symmetrical in the horizontal cross-section of the can. This amounted to the rotation of the imaginary vertical half-plane around the central axis ($x = y$). This assumption was necessary because there was no way of locating the imaginary half-plane except to measure all points in each can. The temperature at the surface of the container was assumed to rise instantaneously to retort temperature. The thermal resistivity of the can,

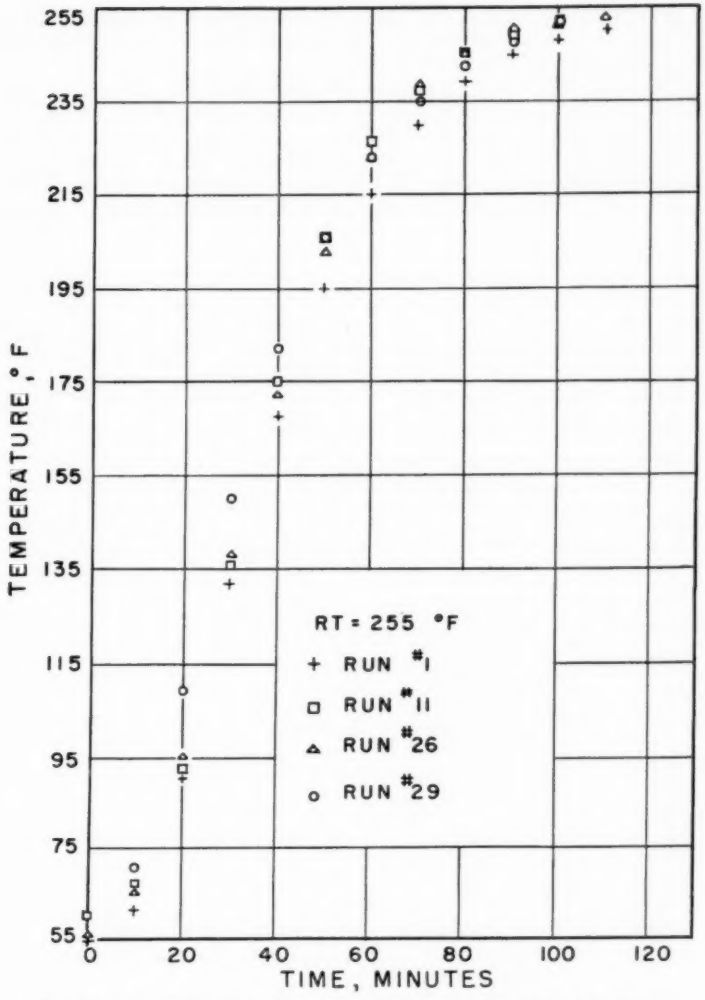

Figure 4. Four replications of heating curves for Location 32 (RT is 255° F.).

the influence of the inside and outside film on the container surfaces and the influence of the liquid surrounding the meat were not measured. It was assumed that the temperature in the retort was instantaneously forwarded to the piece of meat inside the can.

Determination of Thermal Diffusivity. Four temperature determinations corresponding to each time interval were tabulated and averaged to obtain

the mean temperature, (T_m), at any given location in the can as a function of time. This mean temperature was graphed in both rectangular and semi-logarithmic coordinates (Figure 5).

The next step was to determine the function $F(x,y,z,t,T) = 0$. The form of the theoretical relationship was assumed to be correct, because the curves were approximately straight lines in semi-logarithmic coordinates. The theoretical relationship in its first approximation (Eq. 1) was transformed from the exponential $y' = a'e^{b't}$ form into the logarithmic form as follows:

$$\log (RT - CT) = \log[A_{11}(RT - IT)J_0(\mu_1 r)\sin \lambda_1(z + l)] - k(\mu_1^2 + \lambda_1^2) (\log e)t \quad \text{Eq. 2]}$$

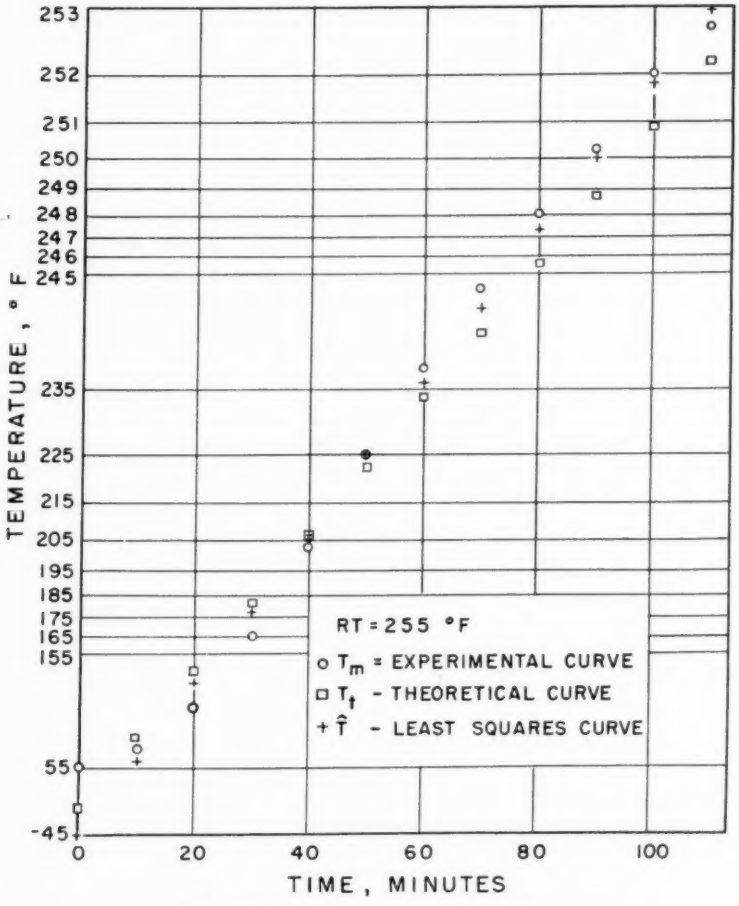

Figure 5. Heating curves for Location 31.

to give the general expression of the time-temperature relationship in the space of the can as follows:

$$\log y' = \log a' + b't \ (\log e) \qquad \text{Eq. 2a}$$

where

$$y' = RT - CT$$
$$a' = A_{11}(RT - IT)J_0(\mu_1 r)\sin \lambda_1(z + l)$$
$$b' = -k(\mu_1^2 + \lambda_1^2)$$

This relationship was simplified to give an equation of a straight line in semi-logarithmic coordinates of the following form:

$$y = a + bt \qquad \text{Eq. 2b}$$

where

$$y = \log y'$$
$$a = \log a'$$
$$b = b'(\log e)$$

where a is the intercept of the line on the y-axis, and b the slope. The values of the apparent thermal diffusivity, k, were determined from the theoretical relationship using the mean experimental data as recorded at 30 and 90 minutes after the processing was started. Calculations were made separately for each location at each temperature and averaged to obtain a mean value

TABLE 1

Apparent thermal diffusivity of beef
(k — sq.cm. per min.)

Location	RT = 225°F.		RT = 255°F.	
	k_{30} min.	k_{90} min.	k_{30} min.	k_{90} min.
32	0.09264	0.10091	0.09454	0.10849
14	0.09388	0.11055	0.10587	0.12600
41	0.08643	0.09539	0.08850	0.10463
24	0.09057	0.09705	0.08817	0.10160
31	0.08701	0.09870	0.08437	0.11001
13	0.08734	0.09795	0.09429	0.10463
43	0.09264	0.10256	0.09206	0.11249
12	0.08983	0.09994	0.09810	0.12462
21	0.06038	0.10146	0.09346	0.11855
42	0.07113	0.09741	0.09371	0.13565
34	0.08230	0.09581	0.07196	0.10388
23	0.08437	0.10489	0.08519	0.10904
$k_{average}$	0.08488	0.09148	0.09085	0.11329
k_{mean}	0.08818		0.10208	
$\hat{k}$	0.10352		0.11320	
variance, s^2	0.0000746			

of k at 225° and 255°F. which could be applied to all the locations tested. These values are tabulated in Table 1.

An analysis of variance was made to determine the effect of processing temperature and the time at which the measurement was made on the value of thermal diffusivity. Both these factors were found to be highly significant which indicates that the term of "apparent" diffusivity is well justified. In this case the diffusivity is actually not constant, as assumed in the theoretical

equation but a function of temperature. The analysis of variance indicated that the processing temperature although very significant itself had a smaller effect on the diffusivity than the time at which the measurement was made. This effect of time is actually a combined effect of time and temperature (at the location tested), as the temperature at a point was increasing with the time. The rise in temperature was faster at the higher processing temperature than at the lower which accounts for the effect of processing temperatures. This would substantiate the contention that the temperature at the point is the main factor in change of diffusivity value, the time being important only indirectly. It may be stated that most likely the diffusivity is not a true constant but varies with the temperature at the location tested. The variation, however, seemed to be rather small except for the initial period of heating, and as it is shown in further computations, could be assumed to be constant for practical purposes and resulted in a relationship found in good agreement with the experimental data.

Comparison between theoretical and regression estimates. The mean thermal diffusivity for each processing temperature was substituted into the general equation (Eq. 2) and after other terms were computed for each location separately, a series of constants in the theoretical heating equations was determined for the locations investigated. These constants are summarized in Table 2.

The equations thus obtained were used to graph the theoretical heating curves by substituting time and computing the corresponding theoretical temperature (T_t), as shown in Figure 5. Thus for every location two heating curves were obtained: one experimental and one theoretical.

TABLE 2

Constants in the theoretical heating equations used for locations distributed within one cylindrical piece of beef sealed in a No. 2 can

RT b' b Location	225°F. 0.03554 0.01543			255°F. 0.04114 0.01786		
	j	a'	a	j	a'	a
32	1.853	169.85	2.2299	1.853	202.45	2.3064
14	1.853	169.49	2.2292	1.853	207.47	2.3170
41	1.815	168.40	2.2263	1.815	201.21	2.3036
24	1.815	171.11	2.2332	1.815	197.06	2.2947
31	1.290	119.50	2.0774	1.290	139.81	2.1455
13	1.290	123.68	2.0924	1.290	144.92	2.1611
43	1.535	138.07	2.1402	1.535	172.32	2.2363
12	1.535	138.15	2.1402	1.535	172.03	2.2356
21	0.688	60.50	1.7817	0.688	76.94	1.8861
42	0.688	64.07	1.8066	0.688	75.28	1.8767
34	1.100	98.89	1.9951	1.100	123.32	2.0910
23	1.417	131.29	2.1183	1.417	155.89	2.1928

$a' = A_{1l}(RT - IT)J_0(\mu_1 r)\sin \lambda_1(z + l)$
$a = \log a'$
$b' = -k(\mu_1^2 + \lambda_1^2) \qquad b = b'(\log e)$
$j = a'/(RT - IT)$

The differences between the means of the observed temperatures, or as transformed, of the logarithms of mean temperature differences ($RT - T_m$) and corresponding values as computed from theoretical considerations were subjected to the least squares treatment, and the regression coefficient, $\hat{b}$ and intercept, $\hat{a}$ in the regression equation were found by treating the differences between the experimental data and theoretical estimates of temperature, rather than by directly computing the regression equation (12 p.241). This method simplified the computations, and thus the regression coefficient, $\hat{b}$ was found as ($b_{theor.} + \Delta b$). This treatment yielded corrections for the slope, b, and for the intercept, a, to give a best fitting curve for each individual location. These corrections, Δa and Δb, are applicable to the line as represented on the semi-logarithmic coordinates. In fact $(\Delta b + b)/b$ results in a correction factor, f_b, for the slope $b' = -k(\mu_1^2 + \lambda_1^2)$ and thus for k as determined previously assuming $(\mu_1^2 + \lambda_1^2)$ to be without error. ($\Delta a = \log f_a$) corresponds directly to a correction factor, f_a, or the theoretical term in the general heating equation; $a' = A_{11}(RT - IT)J_0(\mu_1 r)\sin \lambda_1(z + l)$ which as may be noticed is equal to $j(RT - IT)$.

The values of j are tabulated in Tables 2 and 4.

These corrections, Δa and Δb were computed separately for each location, averaged to obtain a mean correction factor for all the locations at one processing temperature and tabulated in Table 3. The resulting mean

TABLE 3

Regression corrections for the intercept, a, and slope, b, of heating curves presented in semi-logarithmic coordinates

Location	RT = 225°F.		RT = 255°F.	
	$(\Delta a)10^2$	$(-\Delta b)10^2$	$(\Delta a)10^2$	$(-\Delta b)10^2$
32	2.159	2.359	4.887	1.416
14	10.321	5.124	−5.133	2.823
41	2.666	1.302	1.682	0.982
24	1.132	1.535	3.639	0.121
31	9.025	3.051	9.743	2.258
13	6.564	2.302	4.545	0.421
43	3.392	2.633	5.035	1.411
12	−6.721	0.445	19.320	6.183
21	30.561	5.217	25.879	6.447
42	22.300	4.116	30.326	7.447
34	16.981	3.174	17.377	1.386
23	13.703	4.884	12.658	2.480
Average 12	+0.1046	+0.00301	+0.1083	+0.00278
Average 10	+0.0727	+0.00268	+0.0737	+0.00195
Correction factors used to correct a' and b from Table 2		$f_a = 1.182$ $f_b = 1.174$		$f_a = 1.185$ $f_b = 1.109$

$\text{Log } \hat{a}' = \log a' + \Delta a$
$\text{Log } (\hat{a}'/a') = \Delta a$
$\text{Log } f_a = \Delta a$
$f_b = (b + \Delta b)/b = \hat{b}/b$

corrections were applied to the general theoretical relationship and yielded a series of corrected regression constants in the relationship for individual locations. These constants are tabulated in Table 4. This treatment then resulted in average best fitting regression curves for all of the points.

These regression equations again were used to graph heating curves substituting time and computing the corresponding temperatures ($\hat{T}$). One of these curves is shown in Figure 5.

In all these computations the values of Δa and Δb and k were averaged for each temperature range over ten locations only, excluding points 21 and 42 which were farthest from the center and which required excessively large

TABLE 4

Constants in the regression heating equations used for locations distributed within one cylindrical piece of beef sealed in a No. 2 can

RT / b' / b / Location	225°F. 0.04172 0.01811			255°F. 0.04562 0.01981		
	$\hat{J}$	$\hat{a}'$	$\hat{a}$	$\hat{J}$	$\hat{a}'$	$\hat{a}$
32	2.190	200.75	2.3026	2.196	239.95	2.3801
14	2.190	200.40	2.3019	2.196	245.90	2.3907
41	2.145	199.10	2.2990	2.151	238.40	2.3773
24	2.145	202.20	2.3059	2.151	233.55	2.3684
31	1.525	141.30	2.1501	1.529	165.65	2.2192
13	1.525	146.25	2.1651	1.529	171.70	2.2348
43	1.814	163.25	2.2129	1.819	204.20	2.3100
12	1.814	163.25	2.2129	1.819	203.85	2.3093
21	0.813	71.51	1.8544	0.815	91.16	1.9598
42	0.813	75.73	1.8793	0.815	89.21	1.9504
34	1.300	116.90	2.0678	1.303	146.10	2.1647
23	1.675	155.22	2.1910	1.679	184.70	2.2665

For meaning of symbols see Table 2.

corrections. The corrections determined on this basis were applied to all of the locations under investigation. This resulted in a more restricted application of equations subsequently developed.

By graphing the observed mean temperatures and those computed from the theoretical and regression relationships a graphical means was provided for estimating the closeness of fit. Inspection of curves obtained in this way indicated good agreement between the two relationships developed and the experimental data. Without further analysis, however, it would be impossible to express preference for either relationship, or even to contend that either one was applicable to the curves obtained experimentally when considering the entire space in the container. Undoubtedly the corrections as determined for each location individually would give a reliable relationship for the location investigated. The average correction, however, as determined for the whole space would not necessarily result in a better fit nor even in a good one.

Calculation of errors of estimate. In view of the above facts the probable errors of the constants and temperatures in the theoretical and regression relationships were computed with respect to the observed data, and compared with the probable errors estimated from the precision of the measurements made in the course of the experiment. Such an estimate assumes heat transfer in an ideal isotropic body where errors are due solely to the time, temperature, and length measuring devices; it can be made (12, p. 208–14) by differentiation of the theoretical equation with respect to the variable tested. This probable error estimate will be henceforth referred to as "theoretical probable error" in contrast to the probable error estimate by means of standard statistical methods, which shall be referred to simply as "probable error." The probable error, $p = 0.6745\,\sigma$, where σ is the standard deviation. In computing errors the observation made at zero time was neglected since at that time the retort was operating with open vents, and temperatures attained would have very little sterilizing value.

The theoretical probable error in the thermal diffusivity determinations for each individual location was calculated as p_k/k. This error corresponds to $p_{\hat{b}}/\hat{b}$, the probable fractional error in the determination of the slope, $\hat{b}$. The theoretical probable fractional error due to the precision of temperature measurements was calculated using a mean logarithmic temperature difference during heating; $[(RT - IT) - (RT - T_f)] \;/\; \log[(RT - IT)/(RT - T_f)]$, where T_f is the final temperature after 90 minutes of processing. This error corresponds to the probable error, p_y, for the observed values of $\log (RT - T_m)$ because p_T/T is equal to $p_{\log T}$. Standard statistical methods were used to obtain $p_{\hat{y}}$. The precision of measurements has been tabulated in Table 5.

TABLE 5.
Precision of measurements of temperature, time, and length

	T °F	RT °F	r inches	z inches	t seconds
Error, p	±1	±2	± 1/16	± 1/16	±5

The probable errors and the theoretical probable errors are tabulated in Table 6 which offers means for their comparison. The errors were averaged over 10 interior points and over all 12 points separately to obtain their mean values and to compare the difference between the two means. This two-way averaging would ultimately indicate whether the equation developed could be applied to the entire space tested or to the interior locations only.

The average probable errors in temperature determination for the theoretical and regression curves did not exceed the average theoretical probable error at either retort temperature. Only two out of 24 individual locations showed probable regression errors exceeding the theoretical probable errors computed for these locations. Both of them occurred at the 255°F. processing temperature. Smaller errors resulted in the use of the regression technique when cans were processed at 225°F.; no appreciable difference in the magnitude of the errors was observed in the cans which were processed at 255°F. retort temperature. These results indicate that both the theoretical and

TABLE 6

Average probable errors in determination of constants for the theoretical and regression equations and theoretical probable errors estimated from precision of measurements (%)

	Average over 10 interior locations		Average over all 12 locations	
RT (°F.)	225	255	225	255
	%	%	%	%
Probable errors in temp. differences:				
Theoretical Equation, p_{y_t}	11.31	8.82	11.31	10.62
Regression equation, $p_{\hat{y}}$	5.81	8.04	6.09	9.06
Theoretical (from measurements), p_y	12.02	10.43	12.65	11.43
Probable errors in determination of intercept on semi-log paper:				
Theoretical equation, p_{a_t}	6.39	4.98	6.39	5.99
Regression equation, $p_{\hat{a}}$	3.28	3.59	3.44	4.33
Fractional errors in slope and k determination:				
Theoretical equation, p_b/b	6.99	4.71	6.99	5.75
Regression equation, $p_{\hat{b}}/\hat{b}$	3.04	3.86	3.19	4.36
Theoretical (from measurements), p_k/k	8.05	9.08	9.05	10.76

regression curves fell well within the range of observed values. In other words the actual probable errors did not exceed their estimates based on the precision of measurements afforded by the instruments used.

The probable fractional errors in the thermal diffusivity (or slope) determination did not exceed the theoretical probable errors, i.e., both the average of all the theoretical probable errors, and the theoretical probable errors for individual locations were larger than the corresponding probable errors for the theoretical relationship. It should be noted that the value of thermal diffusivity, k, as determined after the correction factors were applied to the theoretical equation for both processing temperatures, is equal to the, k, value as determined for a retort temperature of 255°F. after 90 minutes of processing $\pm 8.62\%$. This is approximately the theoretical probable error.

Development of isothermals and isochronals. The mathematical relationships developed would give means for plotting temperature distribution diagrams at any time after the processing was started, at any point in the can within the region investigated during this experiment, and within the range of processing temperatures from 225° to 255°F.

Approaching the problem from a purely experimental standpoint, it was necessary to graph observed temperature distributions. The heating data for points shown in Figure 2 were used to ascertain the temperatures at points in the space of the can other than those tested during the investigation. Graphs of temperature vs. distance between points located along different lines in the can were made for 10, 20, 30 . . . . . 80 minutes after processing was started. Such curves are shown in Figures 6, 7, 8, and 9. These graphs were

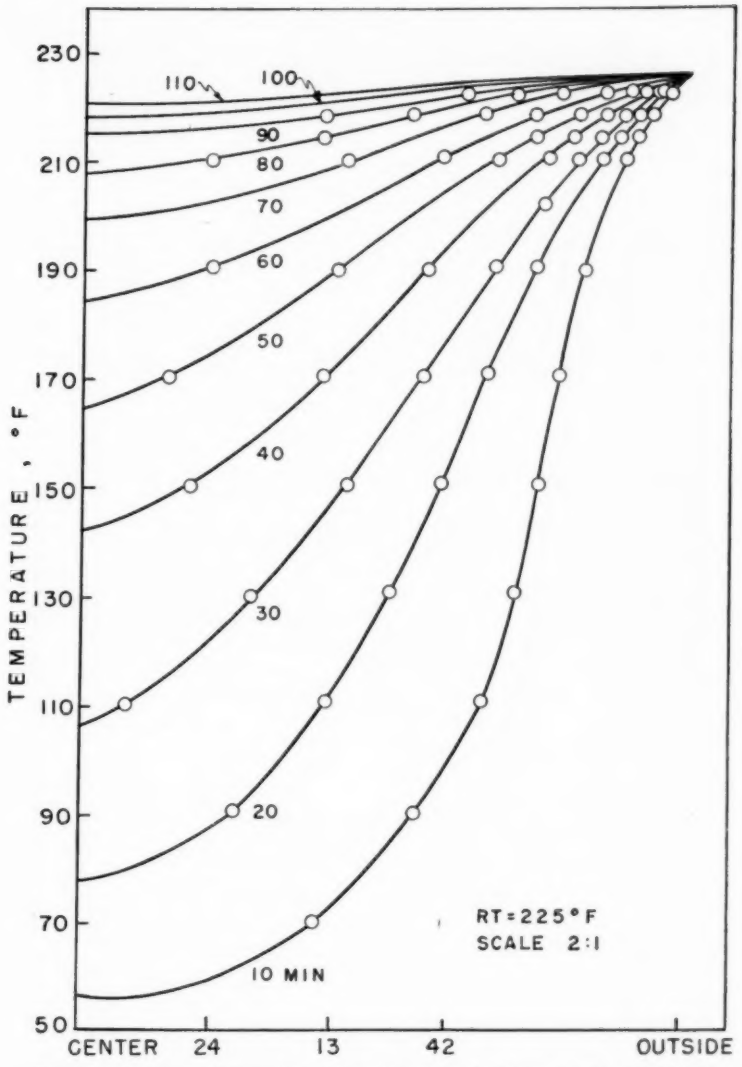

Figure 6. Isochronal temperature distribution at the Locations (24, 13, and 42) along the line from the center to the outside of the can at 10 minute intervals. (Points shown were used for plotting isothermals.)

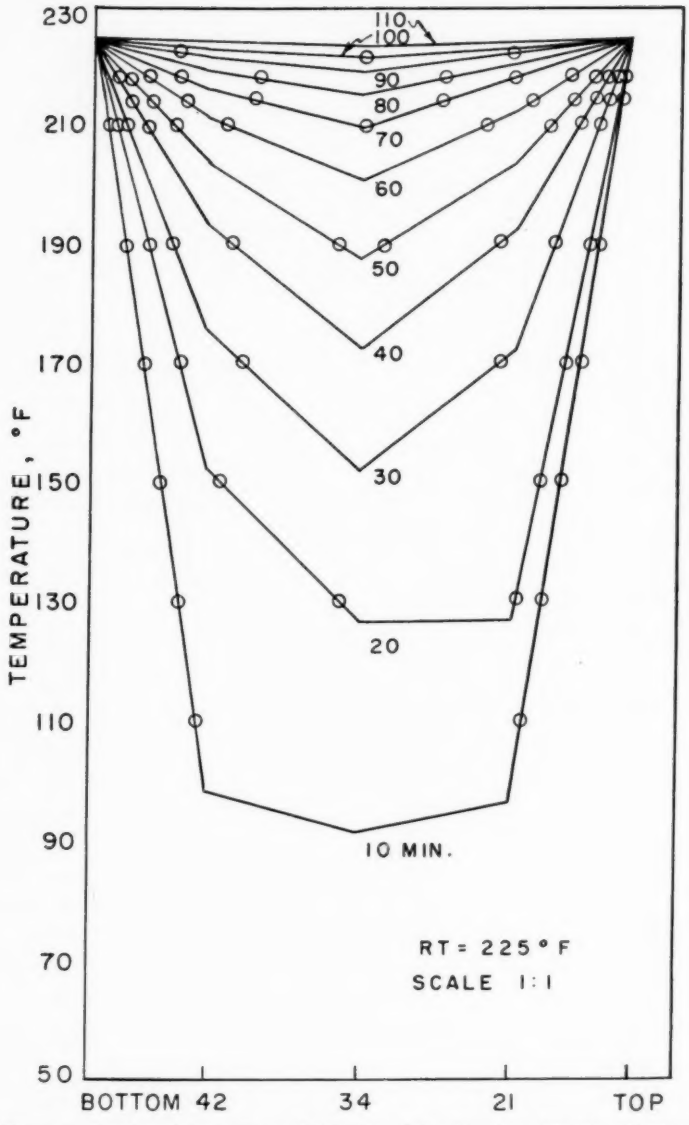

Figure 7. Isochronal temperature distribution at the Locations (42, 34, and 21) on the line from the bottom to the top of the can at 10 minute intervals. (Points shown were used for plotting isothermals.)

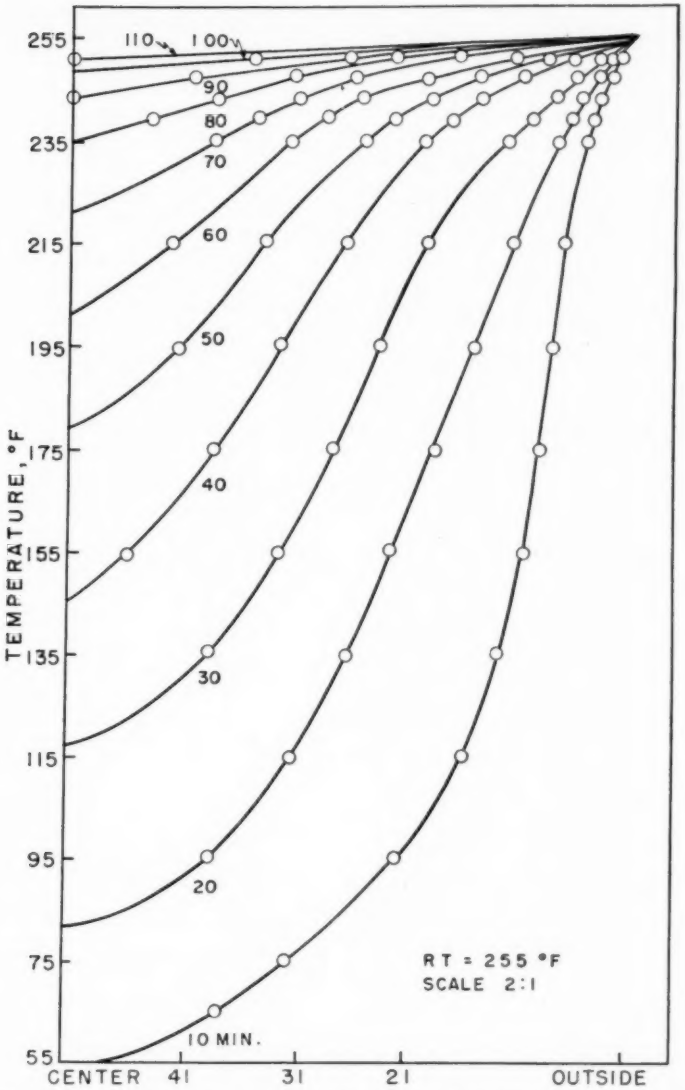

Figure 8. Isochronal temperature distribution at the Locations (41, 31, and 21) on the line from the center to the outside of the can at 10 minute intervals. (Points shown were used for plotting isothermals.)

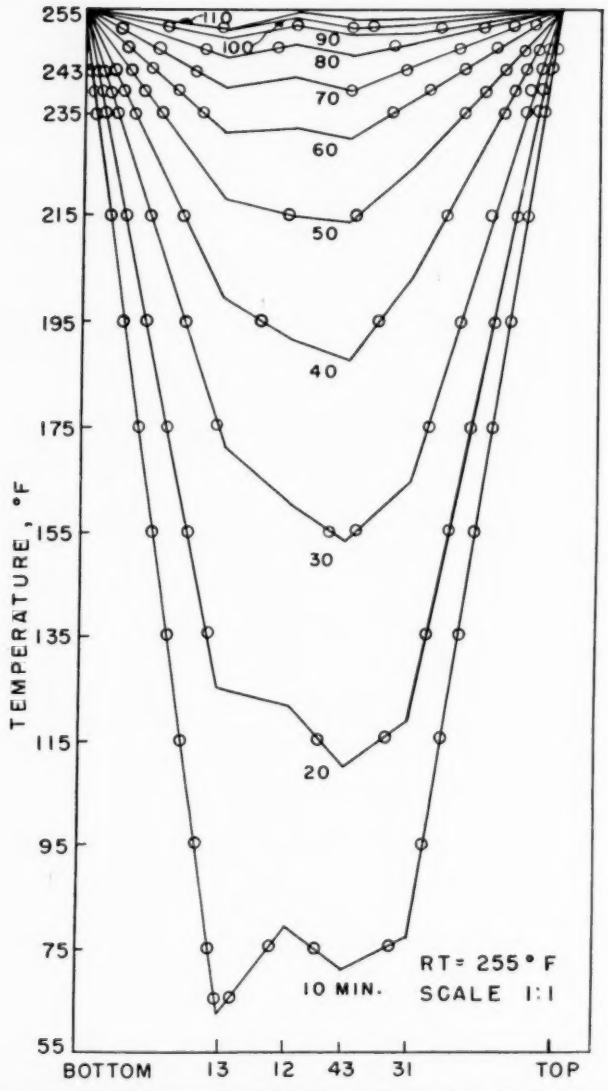

Figure 9. Isochronal temperature distribution at the Locations (13, 12, 43, and 31) located on the line from the bottom to the top of the can at 10 minute intervals. (Points shown were used for plotting isothermals.)

used for interpolation in graphing the isothermal surface distribution diagrams for:

1) distributions of temperatures in the meat at any given time during the heating cycle: (Figures 10 and 11) (isothermals).
2) the movement of an isothermal with the time of processing (Figures 12 and 13) (isochronals).

The horizontal cross-sections were assumed to be circular in shape.

To obtain the lethality of the heating processes, the can was divided arbitrarily into 4 hypothetical cylinders (radii—1.1, 2.2, 3.3 and 4.4 cm.) which roughly approximates the hypothetical "cylinders" formed by the isothermal surfaces (Figure 14). This division of the can space into four cylindrical shells was made in an attempt to modify the thermal process evaluation and shift the basis for such an evaluation from a point estimate to a volume estimate. The volume of each of the hollow cylinders was computed and average temperatures within these cylinders were determined over the heating cycles (Figures 15 and 16). The mean F_0 values for each cylinder were computed from the heating data and a T.D.T. curve with $z = 18$ and $F = 2.78$. Graphical integration was used to obtain F_0 values and these were then graphed against cylinder volume (Figure 17) assuming instantaneous cooling in each case.

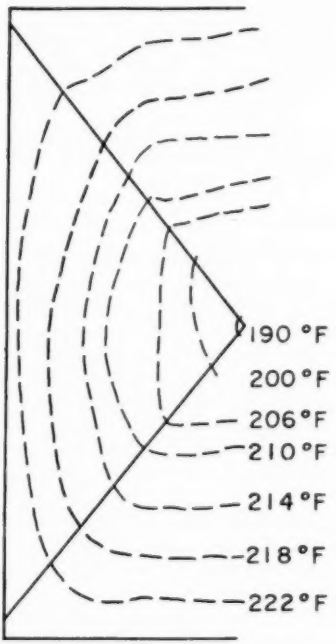

Figure 10. Positions of the 70-minute isothermals (RT is 225°F.).

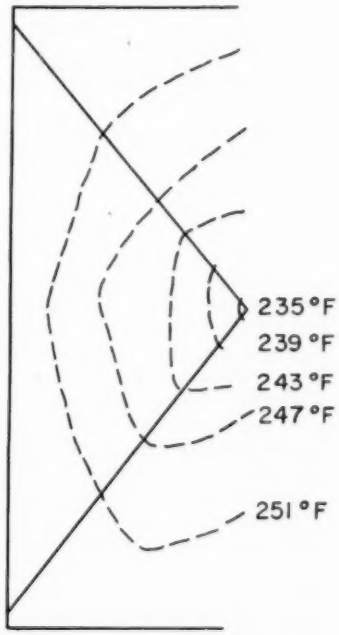

Figure 11. Positions of 80-minute isothermals (RT is 255°F.).

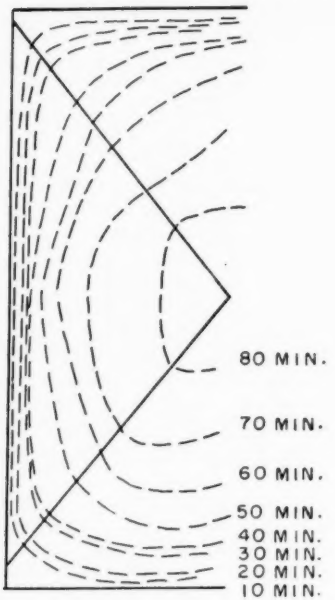

Figure 12. Movement of 243°F. isothermal with time (RT is 255°F. — isochronals).

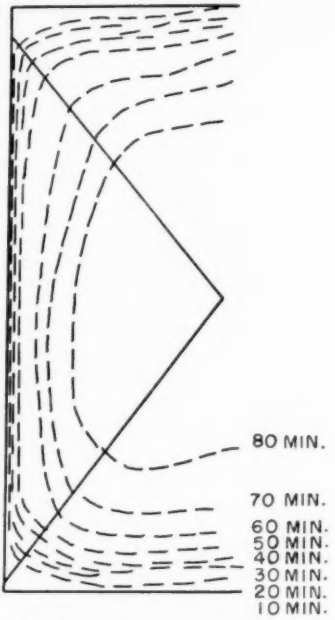

Figure 13. Movement of 218°F. isothermal with time (RT is 225°F. — isochronals).

DISCUSSION

The lack of homogeneity of the meat tested during this experiment has been emphasized. It seems advisable to point out the variations encountered among replications of heating curves recorded for the same location in the container at each processing temperature used. A simple inspection of Figures 3 and 4 shows considerable deviation from the mean displayed by the heating curves for any given run. These figures are representative of 22 other graphs showing quite similar characteristics. In view of these individual variations, the results obtained in the course of the experiment may be considered satisfactory. The conclusions to be drawn from the results obtained can pertain only to average values, and large variations must be expected in each individual case.

As observed from Table 6, the probable experimental errors in the determination of thermal diffusivity, k, and in the observed mean values of temperature fell well within the expected range of precision considering the errors in measurement of temperature, time, and distance during the experiment. This finding implies that either of the relationships used in computation of constants important for the development of an equation suitable for the determination of the temperature distribution could be applied to express the experimental data. The value of the apparent thermal diffusivity as

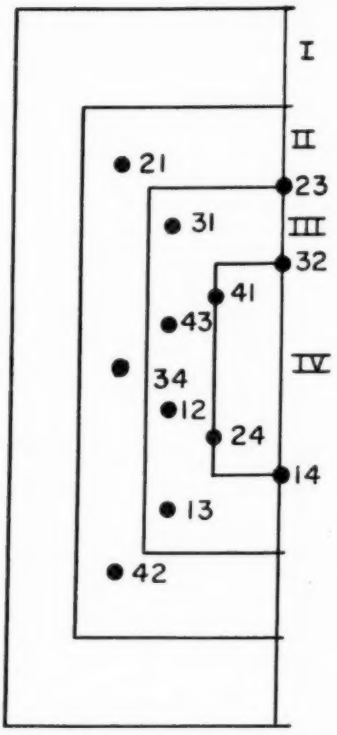

Figure 14. Location of the hypothetical cylinders in the can.

corrected by the use of regression technique was found to be equal within reasonable limits of error to the value as determined after 90 minutes of processing at 255°F.

These results indicate that a combination of the theoretical and regression relationships would be in order to give an equation of best fit to the experimental data. Considering the fact that the regression lines resulted in smaller average errors, it would be desirable to retain some of the corrections it has introduced into the theoretical relationship. By accepting the value of the apparent thermal diffusivity determined at 255°F. after 90 minutes of processing, there is no necessity for correction of the slope of the heating curve or of the exponential term of the theoretical equation. The correction to be retained is the correction for the intercept on the y-axis of the semilogarithmic coordinates which is practically the same for both processing temperatures: Δa_{225} is equal to 0.0727 and Δa_{255} is equal to 0.0737 which gives an average correction factor of 1.18 for the term $A_{11}(RT - IT)J_0(\mu_1 r) \sin \lambda_1(z + l)$. It has been a common practice in thermal process evaluation to correct the formulae for the initial lag of the heating curve. The equation

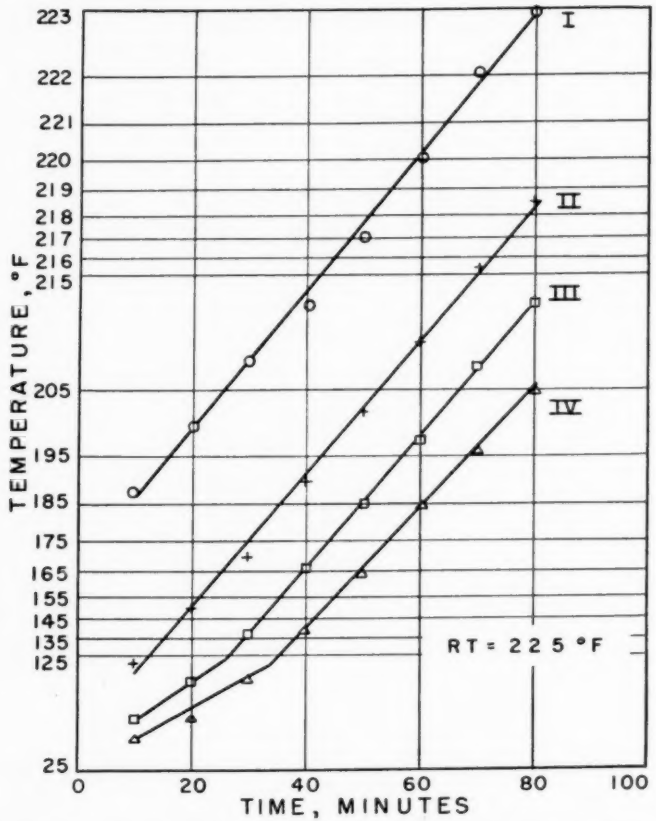

Figure 15. Average heating curves for the hypothetical cylinders (I, II, III, IV) (RT is 225°F.).

to be used in temperature distribution determination and in evaluation of the movement of isothermal surfaces in time and in space for No. 2 cans would assume the following exponential form:

$$CT = RT - 1.18A_{11}(RT - IT)J_0(\mu_1 r)\sin\ \lambda_1(z + l)e^{-k(\mu_1^2 + \lambda_1^2)t} \qquad \text{Eq. 3]}$$

or by substituting constants appropriate for a No. 2 can:

$$CT = RT - 2.407(RT - IT)J_0(0.572\ r)\sin\ 0.275\ (z + 5.8)e^{-0.403kt} \qquad \text{Eq. 3a]}$$

if the measurements are made in cgmin. units.

Inspection of curves and calculations shows that the relationship developed fits the experimental data better in the higher portion of the heating curves, that is after the container reaches higher temperatures. This realm is of great importance in the evaluation of the thermal treatment required for food processing (*9*). Whereas large deviations must be expected from

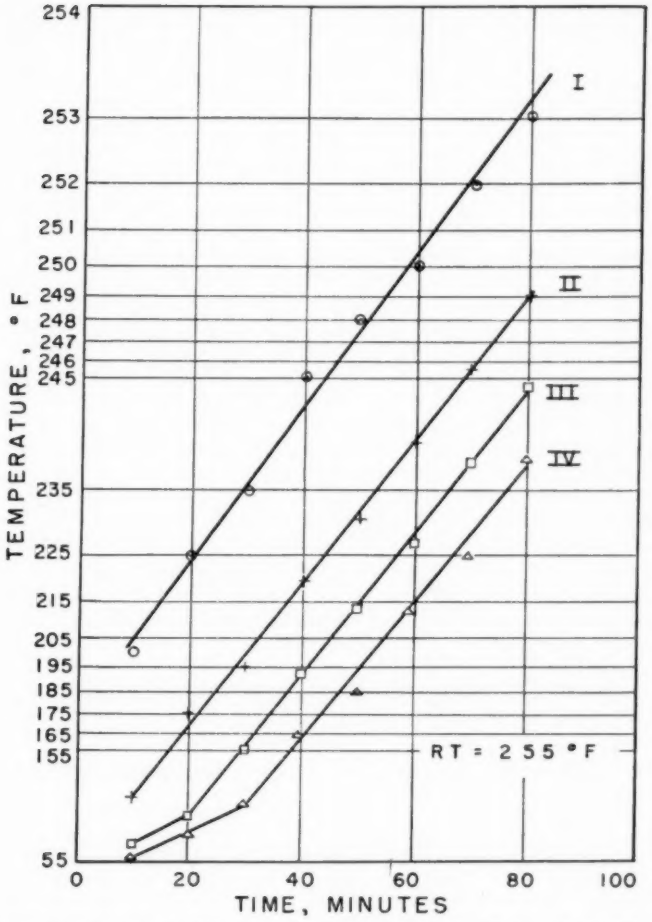

Figure 16. Average heating curves for the hypothetical cylinders (I, II, III, IV) (RT is 255°F.).

Equation 3a in the lower part of the heating curve, experimental evidence indicates that after approximately 25 min. at 225°F. and 15 minutes at 255°F. processing temperatures the experimental data will be in good agreement with the data computed from the equation.

Throughout the evaluation of the experimental data the corrections required for the points farthest removed from the center were consistently larger than for other points in the can. The error in determination of the constants for these points followed the same trend, and although the estimates of error based on precision of the measurements were also larger for these

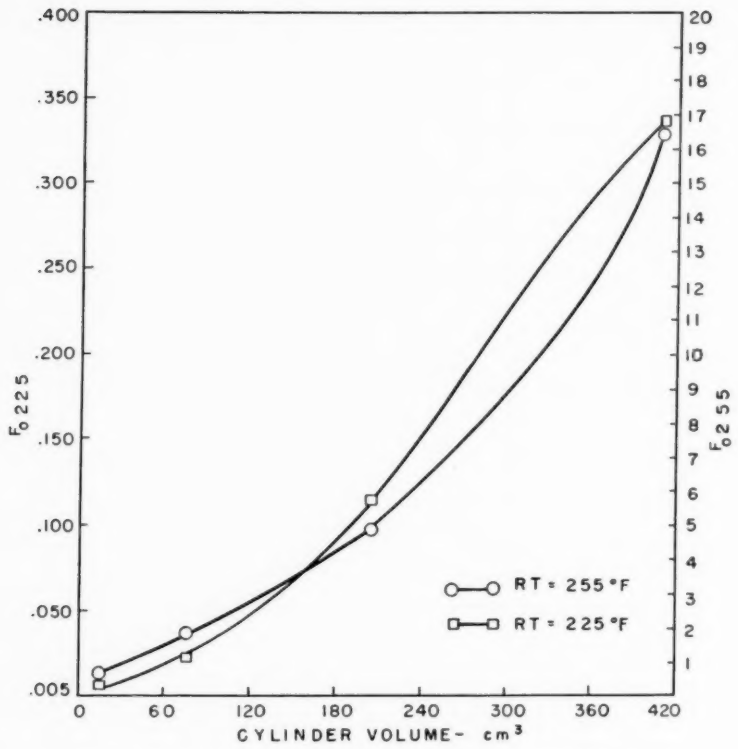

Figure 17. F_0 values as a function of the hypothetical cylinder volume.

points than for the others, this phenomenon seems to indicate that the actual heating rate at these locations, and at the beginning of processing especially, was higher than theoretically expected. Most likely this higher heating rate is due to the influence of convection in the surrounding liquid. As no points closer to the container wall were investigated, no conclusive statement can be made about the influence of convection, especially because the heating curves of these points still fall within the precision range. Therefore, for all of the region investigated it may be said that the developed equation holds.

The form of $F(r,z,t,T) = 0$ indicates the symmetry of the isothermal surfaces with respect to the central vertical axis of the can and with respect to the plane perpendicular to it through the geometric center. The constants for individual locations (Tables 2 and 4) indicate clearly that this condition was approximated very closely in reality. The discrepancies in constants as computed for symetrically distributed corresponding points were due mainly to the differences in initial temperatures, also to the approximation in reading

trigonometric and Bessel functions. The temperature distributions as shown on diagrams drawn directly from the experimental data (Figures 10 and 11) seem to justify the contention of symmetrical distribution.

All conclusions drawn from the evidence collected during this experiment, and all contentions made, have necessarily to be limited to the region of the container investigated and to the range of processing temperatures used. The large errors involved in the determinations, of an order of magnitude as high as 20%, should be kept in mind when using the relationship.

The equation as accepted on the basis of this study provides an expression from which the movement of the isothermal surfaces in time and in space can be determined as well as the temperature distribution patterns at any time. By fixing any three of the four variables involved the fourth can be computed, and thus, if the temperature distribution pattern is desired for a given time, the location of an isothermal surface can be ascertained by substituting values of r, z, and T in the equation.

The experimental temperature distribution diagrams show the movement of isothermals and their distributions within the can. Figure 12 shows the movement of the 243°F. temperature line during processing at 255°F. for 80 minutes. These curves suggest that the temperature distribution pattern, starting from a steady state, where all points in the can have identical temperatures, changes from a cylindrical distribution at the outset to an ellipsoidal at the end of the heating period used. While the same tendency is noted (Figure 13) in the distributions for RT = 225°F. the change is not as pronounced as it is at the higher processing temperature.

Many of the distribution curves exhibit a tendency to be cardioid in shape (Figures 10, 11, 12, and 13) suggesting that the temperatures near the extreme of the vertical axis of the can, vary considerably from those which would be expected if the distributions were either cylindrical or ellipsoidal.

The present accepted procedure is to evaluate the heat penetration data at the center of the container and thus the lethality of the process is a point estimate. By considering volume lethality and bacterial distribution other than uniform throughout the can it may be possible to simplify the process evaluation and to shift the basis for such an evaluation from a point estimate of lethality to the total F_0 effect on the whole contents of the can. This concept could not be very well tested during this experiment since the heating phase only was considered. The investigation of the cooling process would be very important especially in determining the F_0 values for the whole process. The importance of the cooling process was emphasized by Hicks (*5*), and investigation of this process would help in ascertaining the point of lowest lethality. As it was observed the center point continues to heat up after the steam is cut off, especially at higher processing temperatures. There is a possibility that although it is the slowest heating point, it may be subjected to more intensive heat treatment than some other point in the can which cools more rapidly. Evaluation of the required F_0 values by means of the relationship developed by Hicks (*5*) would also be of considerable interest.

The extension of this type of research to higher temperature processes should open some new possibilities in meat processing and in this case the cooling process should assume especial importance.

SUMMARY

An investigation of time-temperature relationships (temperature distributions) occurring in beef sealed in No. 2 cans under the conditions maintained during this experiment showed a good agreement of the experimental data with theoretical expectations. Except for the induction period at the beginning of the process, the heating curves approximated semi-logarithmic straight lines.

Isothermal lines in the vertical half-plane tend to change from approximately cylindrical to approximately ellipsoidal in shape. Deviations along the central axis suggest a cardioid temperature distribution of some isothermals.

Isochronal lines exhibit characteristics similar to those of the isothermals.

The following equation is applicable for determination of the temperature distribution patterns and for determination of the movement of isothermal surfaces with time and in space when the container is heated:

$$CT = RT - 2.407(RT - IT)J_0(0.572\,r)\sin 0.275\,(z + 5.8)e^{-0.403kt}$$

when cgmin. units are used.

The value of the apparent thermal diffusivity, k, to be used in this equation is, as determined after 90 minutes of processing at 255°F., 0.113 sq. cm./min.

Large variations, of the order of $\pm 20\%$, must be expected in determination of temperatures in individual cases.

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TOXICITY AND BODY WEIGHT-DEPRESSING EFFECTS IN THE RAT OF WATER-SOLUBLE COMBINATION PRODUCTS OF GOSSYPOL, GOSSYPOL, AND COTTONSEED PIGMENT GLANDS

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In a recent paper Castillon and Altschul *(2)* described experiments on the toxicity of some new water-soluble combination products of gossypol to aquarium fish. The present investigation deals with the acute oral toxicity and effects of the body weight of the rat of identical and similar combinations of gossypol with glycine, dextrose, and peanut protein. The results of incorporating in the diet various levels of pure gossypol and cottonseed pigment glands are also reported. An understanding of the physiological effects bears direct application to the improvement of the food of man and animals.

METHODS

Samples of untreated cottonseed pigment glands, pure gossypol, and combination products with gossypol were prepared as described *(2, 5)*. Individually caged male rats (150-220 g.) were fasted for 18 hours, with water *ad libitum*, in an air-conditioned animal room maintained at 80° ± 1°F. and ca. 45% relative humidity. Single doses of the test materials were administered by stomach tube as 10% solutions or suspensions in distilled water. All rats had free access to stock diet and water after intubation. The per cent composition of the control and basal ration was: whole ground wheat 21.0, meat and bone scraps 18.0, skim milk 15.0, soybean oil meal 15.0, yellow corn 13.0, lard 10.0, yeast and salt mixture 2.5, liver and glandular meal 2.0, alfalfa leaf meal 2.0, wheat germ oil 1.0, vitamin A and D oil (2250 U.S.P. A, 300 U.S.P. D_3 per g.) 0.5. The surviving rats were weighed daily or 3 times per week for periods of 2 weeks or more. Calculation of the LD_{50} value was made after 1 week by the method of Reed and Muench *(7)*. All gossypol analyses reported in this study were made at the Southern Regional Laboratory by the method of Pons and Guthrie *(6)*. In many cases 4 or more individual analyses were made and the average of these is shown as the gossypol content.

In the 4 subacute toxicity experiments, levels of 0, 0.05, 0.075, 0.10, 0.15, 0.20, 0.30, 0.40, 0.50, 0.536, and 1.34 g. of pure gossypol and levels of 1.34 g. of cottonseed pigment glands containing 21.85% gossypol and 0.6 and 0.9 g. of cottonseed pigment glands containing 33.22% gossypol were added per 100 g. of stock ration and fed *ad libitum* to weanling or young adult male rats. These rats were weighed daily for the first 14 days, and at least thrice weekly thereafter.

RESULTS

The samples of gossypol, untreated cottonseed pigment glands, and gossypol combination products are listed in Table 1 in the order of increasing acute oral toxicity. The LD_{50} value recorded for pure gossypol administered as an aqueous suspension was not significantly different from the LD_{50} value for pure gossypol dissolved in soybean oil. There was poor correlation between the gossypol content of the various samples and their LD_{50} values. For example, Sample F, which contained 68.6% extractable gossypol, was more toxic than 100% gossypol, and Sample D, containing

TABLE 1

Acute oral toxicity and effect on body weight of rat of single doses of cottonseed pigment glands, pure gossypol, and combination products of gossypol

Sample	Description	Extractable gossypol content	LD_{50}	Body weight depression caused by:					
				800 mg./kg.		1600 mg./kg.		3200 mg./kg.	
				Gossypol equivalent of dose	Maximum weight loss	Gossypol equivalent of dose	Maximum weight loss	Gossypol equivalent of dose	Maximum weight loss
		%	*mg./kg.*	*mg./kg.*	%	*mg./kg.*	%	*mg./kg.*	%
A	Gossypol-glycine 1:9	7.2	>6000	58	0	115	0	230	0
B	Gossypol-glycine 1:1	35.2	>6000	282	0	563	0	1126	0
C	Gossypol-dextrose 1:1	43.2	3725	346	−3.3	691	−12.4	1382	−21.0
D	Gossypol-peanut protein 1:1	31.1	3220	249	−4.3	498	−20.9	995	4/7 dead
E	Pure gossypol	100.0	2720	800	−12.2	1600	−24.6	3200	3/5 dead
F	Gossypol-glycine 9:1	68.6	2355	549	−3.5	1098	−16.9	2195	3/4 dead
G	Cottonseed pigment glands (a)	28.6	2170	229	−3.9	458	−11.4	915	4/4 dead
H	Cottonseed pigment glands (b)	33.2	2000	266	−5.3	531	−15.2	1062	4/4 dead
I	Cottonseed pigment glands (c)	21.9	1430	175	−8.3	350	7/8 dead	700	4/4 dead

31.1% gossypol, was more toxic than Samples B and C which contained 35.2 and 43.2% gossypol, respectively. Samples of untreated cottonseed pigment glands (G, H, and I) likewise showed no correlation between their acute oral toxicity and their gossypol content; the one with the lowest gossypol content (I) was much more toxic than the other two. All 3 samples of cottonseed pigment glands, however, despite their low gossypol content, were more toxic to the rat than any of the 5 samples of gossypol combination products containing 7.2 to 68.6% gossypol (A, B, C, D, F) or the sample of pure gossypol (E). These data offer further evidence of lack of correlation between analyzed gossypol content and acute oral toxicity as was originally pointed out for the rat by Eagle *et al. (3, 4, 5)* and subsequently confirmed by Ambrose and Robbins *(1)*.

On the other hand, there was better correlation between the extractable gossypol content of the samples and the loss in body weight. The body weight depressions recorded represent the maximum average body weight loss in a group of at least 4 rats observed for periods of 14 to 28 days after administration by stomach tube of the single dose indicated, and are expressed as per cent deviations from the average starting weight of the same group. Since the control rats were gaining weight in the meantime, the maximum deviation from the average body weight of the controls would have been considerably greater than the values indicated. The above results were confirmed by 50 growth curves obtained after intragastric administration of a total of 101 assorted dose levels of these materials to 544 rats. The high mortality caused by the other 51 dose levels precluded the plotting of body weight curves for the latter.

In Table 2 a total of 46 individual doses of water-soluble gossypol combination products, pure gossypol, and untreated cottonseed pigment glands are arranged in the order of increasing maximum average depression of body weight. For any particular material the per cent loss of body weight resulting from a single dose increased as the gossypol equivalent of the dose increased. When all the gossypol equivalents are considered, regardless of the compounds involved, however, the correlation of gossypol equivalent dosage with magnitude of body weight depression is not consistent. In fact, Sample B, despite a gossypol content of 35.2%, caused no depressing effect on body weight at any of 6 gossypol equivalent levels varying from 282 to 2112 mg./kg. In contrast, the maximum average body weight depressions caused by 600, 1200, 1400, 1600, and 2000 mg./kg. of pure gossypol were, respectively, 13.8, 18.7, 23.7, 24.6, and 24.0% below the average starting weight of the group involved. It is difficult to explain why such high doses of gossypol-glycine (1:1) up to and including 6000 mg./kg. (which had a gossypol equivalent of 2112 mg./kg.) failed to cause any adverse effect on body weight, whereas much smaller single doses of gossypol-dextrose (1:1), gossypol-peanut protein (1:1), pure gossypol, gossypol-glycine (9:1), and 3 samples of untreated cottonseed pigment glands caused such very marked depressions of the body weight of rats as indicated in Table 2.

It was found that gossypol-equivalent doses from cottonseed pigment glands (Samples G, H, and I) generally caused greater body weight depressions than were caused by similar gossypol equivalents from the

TABLE 2

Effect of single doses of cottonseed pigment glands, pure gossypol, and gossypol combination products on the body weight and survival of rats

Sample	Dose given	Gossypol equivalent of dose	Mortality ratio	Maximum body wt. depression
	mg./kg.	*mg./kg.*	*dead/dosed*	%
D	50	16	1/5	0
D	100	31	0/5	0
A	800	58	0/4	0
D	200	62	0/5	0
A	1600	115	0/4	0
A	3200	230	0/4	0
B	800	282	0/4	0
A	4000	288	0/4	0
B	1600	563	0/4	0
B	3200	1126	0/4	0
B	4000	1408	0/4	0
B	5000	1760	0/4	0
I	400	88	0/4	−1.7
A	6000	432	0/4	−2.0
A	5000	362	0/4	−2.2
B	6000	2112	0/4	−2.7
D	300	93	0/4	−3.3
C	800	346	0/4	−3.3
D	600	187	0/4	−3.4
F	800	549	0/4	−3.5
G	1000	286	0/4	−3.9
D	800	249	0/4	−4.3
H	1000	332	0/4	−4.5
D	1000	311	0/5	−5.2
H	800	266	0/4	−5.3
I	800	175	0/4	−8.3
E	800	800	0/8	−12.2
G	1200	343	0/4	−12.3
C	1600	691	0/4	−12.4
E	600	600	0/8	−13.8
H	1400	465	0/4	−14.0
G	2000	572	2/6	−14.5
H	1600	531	0/4	−15.2
I	1200	263	0/4	−16.4
G	1800	515	4/10	−16.5
F	1400	960	0/4	−16.9
F	1600	1098	1/8	−16.9
D	1400	435	0/4	−17.8
E	1200	1200	0/4	−18.7
D	1600	498	0/4	−20.9
C	3200	1382	2/7	−21.0
D	2200	684	0/4	−22.8
E	1400	1400	0/4	−23.7
E	2000	2000	0/4	−24.0
E	1600	1600	0/4	−24.6
D	3000	933	1/7	−24.7

various gossypol combination products. The inclusion of higher gossypol equivalent levels from doses of untreated cottonseed pigment glands was limited by the mortalities caused by the greater toxicity of cottonseed pigment glands over the gossypol-combination products or pure gossypol, despite the lower gossypol content of the former.

Subacute Toxicity Experiments

The results obtained in 4 different experiments in which various levels of pure gossypol and cottonseed pigment glands were incorporated in the stock diet are given in Table 3. In Experiment I levels of 0.536 and 1.34% pure gossypol and 1.34% cottonseed pigment glands — equivalent to 0.293% pure gossypol — all caused 100% mortality in weanling rats within 9 days. In Experiment II a level of 0.05% pure gossypol caused no appreciable depression in the body weight of young adult rats, but higher levels caused a definite body weight depression, proportional to the level of pure gossypol added to the diet. Levels of 0.3% and higher of added gossypol caused immediate weight loss, so that the average body weight remained below the average starting weight throughout the experiment.

In Experiment III the diets containing various low levels of added pure gossypol showed little effect on body weight of weanling rats until the 0.1% level was reached. A level of 0.15% of added gossypol caused very marked weight loss. These depressions maintained a similar pattern for a period of 4 weeks, after which all groups were placed on the stock ration to determine the extent of recovery. The average body weights for the groups previously fed 0, 0.05, 0.075, 0.10, and 0.15% gossypol for 4 weeks and subsequently fed the stock ration for 6 weeks were, respectively, 351, 357, 323, 328, and 304 g.

Experiment IV was designed to compare the effect of a level of 0.2% pure gossypol added to the control diet with a level of 0.2% gossypol contributed by adding cottonseed pigment glands of known gossypol content (33.22%). The diet containing the latter caused a much greater average body weight depression than was brought about by a level of 0.2% added pure gossypol. This greater effect, therefore, must be attributed to some other factor or factors in cottonseed.

The diet containing 1.34% of added cottonseed pigment glands (Expt. I) having an equivalent of 0.293% gossypol caused greater effect (mortality, body weight depression) than the diet containing 0.9% added cottonseed pigment glands (Expt. IV) which had a gossypol equivalent of 0.3%, showing again that the greater toxicity of cottonseed pigment glands cannot be explained solely on the basis of their gossypol content.

SUMMARY

Acute oral toxicity of water-soluble gossypol combination products with glycine, dextrose, or peanut protein in the rat is not proportional to the gossypol content.

Depressions in body weight caused by single doses of any one of these gossypol combinations are proportional to the gossypol equivalent administered, but as a group the different samples vary in their effect on body weight in a manner unrelated to the gossypol equivalents of the doses administered.

All water-soluble combination products of gossypol tested were less toxic to rats than cottonseed pigment glands—even when samples of the former had higher extractable gossypol content than the latter.

Untreated cottonseed pigment glands show poor correlation between their extractable gossypol content and their acute oral toxicity or effect on the body weight of the rat, indicating that other factors are involved.

Subacute toxicity tests on rats fed 10 different levels of pure gossypol showed that the body weight depression caused by gossypol is proportional

TABLE 3

Summary of data obtained in feeding experiments

Diet	Gossypol content of diet	No. rats per group	Mean body weight after following days: 0	2	4	6	8	10	12	14	21	28
			g.	g.	g.	g.	g.	g.	g.	g.	g.	g.
Experiment I												
Stock diet control	0	4	49	57	68	80	94	109	122	133	178	218
Stock diet containing 1.34% added CPG C	0.293	4	49	45	41	38	*					
Stock diet containing 0.536% added gossypol	0.536	4	49	44	39	#						
Stock diet containing 1.34% added gossypol	1.340	4	49	41	●							
Experiment II												
Stock diet control	0	5	171	182	191	206	213	221	231	242		
Stock diet containing 0.05% added gossypol	0.05	5	171	179	188	203	212	220	231	239		
Stock diet containing 0.10% added gossypol	0.10	5	171	182	186	199	205	211	216	225		
Stock diet containing 0.20% added gossypol	0.20	5	171	176	179	186	185	187	191	190		
Stock diet containing 0.30% added gossypol	0.30	5	170	169	163	161	157	156	154	151		
Stock diet containing 0.40% added gossypol	0.40	5	170	162	151	145	141	139	136	135		
Stock diet containing 0.50% added gossypol	0.50	5	170	158	146	138	133	132	129	129		
Experiment III												
Stock diet control	0	10	60	68	79	91	100	112	124	135	173	212
Stock diet containing 0.050% added gossypol	0.050	5	59	69	81	95	105	120	130	144	180	217
Stock diet containing 0.075% added gossypol	0.075	5	60	67	77	87	96	109	119	131	161	192
Stock diet containing 0.100% added gossypol	0.100	5	60	67	77	87	97	110	119	130	160	186
Stock diet containing 0.150% added gossypol	0.150	5	60	66	73	80	85	99	101	107	123	142
Experiment IV												
Stock diet control	0	5	55	65	76	86	98	111	118	130	168	203
Stock diet containing 0.2% added gossypol	0.2	5	55	60	69	79	86	99	106	114	139	160
Stock diet containing 0.6% CPG b added	0.2	5	55	57	60	61	65	72	75	77	89▲	95▲
Stock diet containing 0.9% CPG b added	0.3	5	55	53	55	54	53	53	■			

* Two died on 7th day, 1 died on 8th day; all 4 dead within 9 days

\# Two died on 6th day, 1 died on 7th day; all 4 dead within 8 days

● Three died on 4th day; all 4 dead within 5 days.

▲ One died on 17th day; average weights for 4 rats.

■ Two died on 11th day, 1 died on 13th day, 1 died on 14th day; 4/5 dead within 28 days.

to the amount added to the diet. The greater mortality and body weight effects caused by adding various levels of cottonseed pigment glands to the diet cannot be attributed to their gossypol content alone, indicating again that other factors in cottonseed pigment glands are involved.

Acknowledgment

The authors are indebted to members of the Southern Regional Research Laboratory, U. S. Department of Agriculture, for the samples of cottonseed pigment glands, pure gossypol and gossypol combination products, and for the gossypol analyses.

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LINOLEATE OXIDATION CATALYZED BY HOG MUSCLE AND ADIPOSE TISSUE EXTRACTS

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Oxidative rancidity during storage is a serious problem in the refrigerated and frozen preservation of pork products. The oxidation of the unsaturated fats is primarily responsible for the loss of pink color, development of unpleasant odor and flavor, and loss of vitamins. Myoglobin and hemoglobin are co-oxidized in the presence of oxidizing fats and the pink color of pork becomes grey brown *(22)*. Aldehydes formed by the decomposition of fat peroxides are responsible for the unpleasant odor and flavor *(15)*. The essential fatty acid, linoleic acid, comprising about 6% of the fatty acids in pork is readily oxidized and its vitamin activity is lost. Other easily oxidized vitamins may be destroyed in coupled oxidations. These include: vitamin E, vitamin A, vitamin D, pantothenic acid, pyridoxine, and biotin *(6)*.

Lea *(14)* was the first to suggest that an enzyme might be responsible for the rapid fat oxidation in bacon. The only enzyme known to cause the direct oxidation of unsaturated fats is lipoxidase which has been isolated from soybeans *(20)* and is known to occur in many plants *(4)* and in bacteria, as reported by Jezeski *(12)* and earlier workers. In recent years the properties of soybean lipoxidase have been widely investigated *(11)*. Watts and Peng *(21)* examined the catalytic activity of hog muscle extract and concluded that myoglobin and hemoglobin were the catalysts responsible for fat oxidation. The catalytic activity of hemin, hemoglobin, and myoglobin for fat oxidation has been well studied *(2, 9)*. These catalysts could be responsible for the oxidation of pork fat. Reiser *(16)* in 1949 again suggested that an enzyme must play a role because his preparations of hemoglobin-free bacon adipose extracts were found to contain a heat labile catalyst for the oxidation of lard.

It is the purpose of this study to present more conclusive evidence for the presence or absence of lipoxidase or similar enzymes in pork tissues and to show which catalysts are responsible for pork fat oxidation.

METHODS

Pork tissue extracts. Pork loin, cured smoked bacon, and cured smoked ham were obtained from commercial outlets. Uncured bacon and ham were obtained from freshly slaughtered animals. Muscle extracts were prepared by dicing lean tissue and disintegrating in a blendor with a double weight of distilled water for 2 to 3 minutes. The resulting mixture was subject to centrifugation at 850 × gravity to produce 3 layers: a top layer containing most of the fat, a clear aqueous red-colored extract, and a bottom layer of insoluble tissue debris.

Determination of the catalytic activity of the 3 fractions on an equal weight basis indicated that the top layer had about ⅓ the activity of the clear extract, and the bottom layer had the same activity as the clear extract. The equivalent hemin content of the aqueous extract averaged 0.15 mg. per ml. for pork loin as determined by the

alkaline pyridine hemochromogen method *(16)*. The aqueous extract was stored in small vials at −18°C. until ready for use. Dilutions, as necessary, were made with distilled water immediately before use.

Extracts of adipose tissue were made by the method of Reiser *(16)* but could not be freed entirely of occult hemoglobin as he describes.

Occult hemoglobin was determined by the phenolphthalein test *(8)*. The concentration of heme compounds was determined by the alkaline pyridine hemochromogen method and expressed in terms of equivalent hemin content.

Lipoxidase preparation. A crude lipoxidase solution was obtained by aqueous extraction of defatted soybean meal as previously described *(19)*. The potency of the lipoxidase preparation was 30 units per ml. as assayed by the method of Bergström and Holman *(4)*.

Preparation of linoleate substrates. Highly purified linoleic acid was obtained from the Hormel Institute, Austin, Minnesota. The heterogeneous or colloidal linoleate consisted of 7.1×10^{-3} M. linoleic acid neutralized with sodium hydroxide and dissolved in 0.1 M. phosphate buffer at pH 7.0. The homogeneous linoleate consisted of 9×10^{-4} M. sodium linoleate dissolved in 0.1 M. ammonium hydroxide-ammonium chloride buffer at pH 9.0. The linoleate substrates were protected from oxidation during preparation by an atmosphere of nitrogen and during storage by temperatures of −18°C. and an atmosphere of nitrogen.

Oxygen absorption measurements and determination of initial reaction rates. Oxygen absorption measurements were made by conventional techniques using a refrigerated Warburg apparatus. Three ml. of buffered linoleate substrate were placed in the main compartment and 1 ml. of diluted catalyst was placed in the side arm to be mixed with the contents of the main compartment after temperature equilibrium.

In order to make certain that the oxygen absorption was a true measure of linoleate oxidation and not due to oxidation of some component of the tissue extract, spectral absorption measurements of the oxidized linoleate were made. For ethyl linoleate peroxide the molecular extinction coefficient at 232.5 mμ has been reported as 22,700 *(5)*. The shape of the absorption spectrum and a molecular extinction coefficient of 23,000 at 232.5 mμ indicated that linoleate peroxide was the oxidation product which was formed during these oxygen absorption experiments. Hemin, myoglobin, and hemoglobin are also oxidized during the reaction when they are present as catalysts. The amount of oxygen necessary for oxidation of the small concentrations of the heme compounds used in these studies does not cause significant errors in determination of initial reaction rates. Initial reaction rates were determined from the slope of the initial straight line portion of oxygen absorption vs. time plots constructed from at least 5 experimental points.

RESULTS AND DISCUSSIONS

The effect of temperature on the rate of linoleate oxidation. These studies were made at temperatures ranging from 0°C. to 20°C. in order to simulate storage conditions as nearly as possible. The initial reaction rates of linoleate oxidation catalyzed by pork loin muscle extract containing the equivalent of 0.16 mg. hemin per ml. were 75 cmm O_2 per hour, 117 cmm O_2 per hour, and 58 cmm O_2 per hour for the temperatures 0°C., 10°C., and 20°C., respectively. It is interesting that the rate of catalyzed oxidation is greater at 0°C. and 10°C. than at 20°C. This is probably due to the fact that the rate of oxidation decreases less with temperature than does the inhibitory effect of the antioxidants naturally present in the pork extract. At 20°C. the oxidations characteristically showed a pronounced induction period, usually about 2 hours in length; this is also a typical effect of the antioxidants.

The heat lability of muscle and adipose extracts. It has been previously reported that pork muscle and adipose extracts contain heat labile fat oxidation catalysts. Lea *(14)* proposed that the heat lability of pork muscle

extracts may be due to the presence of the enzyme lipoxidase. In the present study temperatures of 50°C. and 80°C. were used to determine the relative lability of pork tissues compared to that of a known preparation of soybean lipoxidase. The lipoxidase enzyme preparation was completely denatured by holding at 80°C. for 2 minutes but could be held for 30 minutes at 50°C. without a significant loss in activity. As shown in Figures 1 and 2 the pork muscle extract which was held at 80°C. for 30

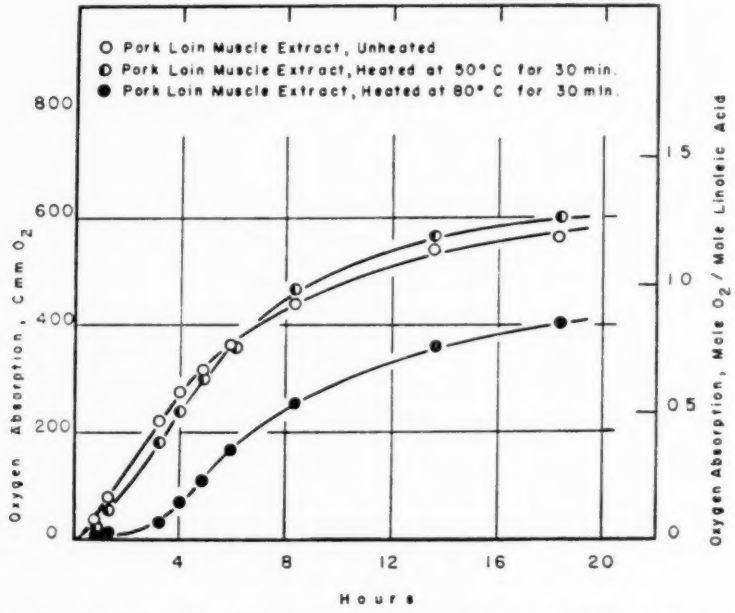

Figure 1. The effect of heating pork muscle extracts upon the catalysis of linoleate oxidation at 0°C.

minutes showed a considerable loss in catalytic activity compared to an identical amount of unheated extract. On the other hand, the pork muscle extract held at 50°C. for 30 minutes did not cause much reduction in the amount of catalysis of linoleate oxidation. These experiments clearly indicate the possibility that the presence of an enzyme like lipoxidase in pork tissues could offer an explanation for the heat lability of the fat oxidation catalysts. In studies related to the catalytic activity of hog muscle extracts Watts *(7, 21)* indicates another possible explanation for this type of heat lability. When the muscle proteins are heated, a coagulation of hemoglobin and myoglobin may occur, and hence the loss of the catalytic effect of the heme portion of these proteins may be due to an effective removal of heme from contact with molecules of unsaturated fats.

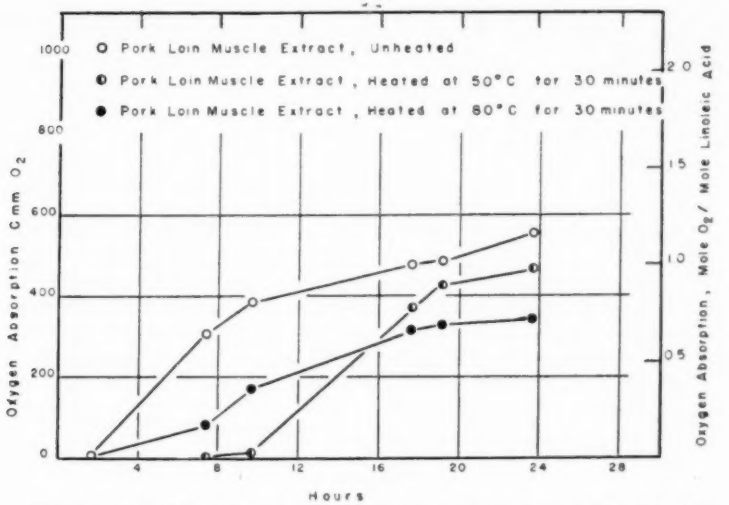

Figure 2. The effect of heating pork muscle extracts upon the catalysis of linoleate oxidation at 20°C.

In this study the explanation of Watts was put to an experimental test. Crystalline hemin was added to an extract of pork muscle and the mixture of the 2 was held at 80°C. for 30 minutes. By appropriate determinations of the rate of linoleate oxidation catalyzed by the mixture of hemin and extract, before and after heating, it was found that 17% of the catalytic effect of the crystalline hemin added was lost during heating. Since hemin itself is heat stable, the loss of catalytic activity is probably due to an effective removal of hemin by protein coagulation even though the entire unfiltered mixture of hemin and extract was used in the determinations of the rate of linoleate catalyzed oxidation.

Thus, on the basis of these observations it would be erroneous to assume that the heat lability of the catalysts present in pork muscle extracts indicates the presence of an enzyme.

An interesting phenomenon shown in Figures 1 and 2 is the induction period caused by heating pork muscle extracts at 50°C. for 30 minutes. Only in the linoleate oxidation carried out at 20°C. is the induction period pronounced. The probable explanation for this inhibition of catalysis is that the heating causes a release of antioxidants or a formation of other reducing substances from the muscle extract constituents. An increase in antioxidant concentration in the pork muscle extract held at 50°C. would show a more pronounced effect in inhibiting linoleate oxidation carried out at 20°C. than at the lower temperature because the antioxidants have greater inhibitory power at higher temperatures.

Reiser *(16)* found that cured bacon adipose tissues freed of hemoglobin would catalyze the oxidation of fats. Since the catalyst in this extract was heat labile he suggested that bacon adipose probably contains an enzyme

able to bring about catalysis of fat oxidation. An attempt to repeat these observations was made in this laboratory, but neither cured nor raw bacon adipose extracts could be completely freed of occult hemoglobin as judged by the very sensitive phenolphthalein test. The heme pigments could be reduced, however, to levels not detectable by the alkaline pyridine hemochromogen method. Uncured bacon adipose extract held at 80°C. for 2 minutes showed no loss of catalytic activity in oxidizing colloidal linoleate—indicating that an enzyme having properties similar to soybean lipoxidase is not one of the active catalysts in bacon adipose.

The absence of lipoxidase in pork tissues. Past attempts to demonstrate the presence or absence of lipoxidase or a similar enzyme in pork tissues have been inconclusive due to the difficulty of separating fat oxidation catalyzed by heme compounds from fat oxidation catalyzed by enzymes. By using a hemogeneous linoleate substrate it is possible to assay the lipoxidase content of tissues containing heme catalysts such as hemoglobin, myoglobin, or hemin. This technique is based on the observation that hemin catalyzes the oxidation of linoleate only in heterogeneous or colloidal systems *(10)*. Lipoxidase, and presumably any other enzyme that would catalyze the oxidation of linoleate, is active in both homogeneous and heterogeneous systems. In fact soybean lipoxidase will catalyze the oxidation of linoleate more effectively at high pH values, principally because of the solubilization of linoleate under these conditions *(18)*.

Using both the heterogeneous linoleate and the homogeneous linoleate preparations described previously, various samples of pork tissue extract have been examined for the catalytic oxidation of linoleate. These results are tabulated in Table 1. Trials with crystalline hemin as catalyst indicate that hemin will cause only slight but somewhat variable oxygen absorption in homogeneous linoleate. With very dilute soybean lipoxidase as catalyst there is a large initial reaction rate of oxygen absorption. Furthermore, when crystalline hemin and lipoxidase are mixed, the catalysis is much greater than with lipoxidase alone. This increased rate of oxygen absorption may be due to the fact that hemin causes the breakdown of the linoleate peroxides formed by lipoxidase and by this mechanism accelerates the autocatalytic oxidation of linoleate. It is also possible that hemin is rapidly co-oxidized in the lipoxidase-linoleate system. Contrary to this observation of increased catalysis, Kies *(13)* reported that hemin completely inhibits lipoxidase catalysis. If soybean lipoxidase is added to an extract of uncured bacon adipose tissue the mixture of the two has pronounced catalytic power. This shows that the bacon adipose extract, which is not capable of catalyzing the oxidation of homogeneous linoleate, does not contain an inhibitor capable of preventing lipoxidase catalysis. Thus, the possibility of an inhibitor completely neutralizing the catalytic ability of an enzyme which could be present in pork is of negligible concern. From these experiments it appears that if lipoxidase, or any enzyme capable of direct oxidation of homogeneous linoleate, were present in a concentration equivalent to 0.05 unit lipoxidase per gram tissue or more, its presence could be easily detected. The data shown in Table 1 give good evidence that such an enzyme is not present in any significant quantity in the various pork tissues assayed.

TABLE 1

Rate of linoleate oxidation catalyzed by hemin, lipoxidase, and pork tissue extracts

Catalyst	Concentration of catalyst under assay conditions	Initial reaction rate Cmm O_2 per hour	
		Homogeneous linoleate	Colloidal linoleate
Crystalline hemin	2×10^{-2} M.	0 to 2.1	76
Lipoxidase	0.025 unit per ml.	43	6
Lipoxidase + hemin	0.037 unit per ml. + 1×10^{-2} M.	102	....
Lipoxidase + uncured bacon adipose	0.025 unit per ml. + 0.25 g. per ml.	45	....
Lipoxidase + uncured bacon adipose	0.050 unit per ml. + 0.20 g. per ml.	53	....
Pork loin muscle No. 1	0.042 g. per ml.	1.8	35
Pork loin muscle No. 2	0.031 g. per ml.	1.8	29
Pork loin muscle No. 3	0.042 g. per ml.	2.1	54
Pork loin adipose	0.042 g. per ml.	1.8	28
Cured bacon muscle	0.07 g. per ml.	0.5	41
Cured bacon adipose	0.25 g. per ml.	0.5	36
Cured ham muscle	0.13 g. per ml.	1.0	23
Cured ham adipose	0.25 g. per ml.	0.5	68
Uncured bacon muscle	0.13 g. per ml.	0.7	96
Uncured bacon muscle	0.042 g. per ml.	0.7	32
Uncured bacon adipose No. 1	0.25 g. per ml.	0.8	28
Uncured bacon adipose No. 1	0.20 g. per ml.	3.2	25
Uncured bacon adipose No. 1	0.15 g. per ml.	2.4	21
Uncured bacon adipose No. 1	0.10 g. per ml.	2.4	16
Uncured bacon adipose No. 1	0.05 g. per ml.	1.6	8
Uncured bacon adipose No. 2	0.25 g. per ml.	1.5	34
Uncured bacon adipose No. 2	0.20 g. per ml.	1.5	....
Uncured bacon adipose No. 2	0.15 g. per ml.	1.5	....
Uncured bacon adipose No. 2	0.10 g. per ml.	2.0	....
Uncured bacon adipose No. 2	0.05 g. per ml.	2.0	....
Protein precipitating from 8 liters of pork loin extract	0.25 g. per ml.	0.7	39

In order to examine more critically the possibility that lipoxidase occurs in pork, the protein fraction precipitating from 8 liters of pork loin extract under conditions which are known to precipitate soybean lipoxidase *(4)* was examined for its ability to catalyze linoleate oxidation by the same technique. By this method also, the experimental evidence tabulated in Table 1 shows the absence of lipoxidase.

The mechanism of catalysis in bacon adipose tissue. It is well known that the oxidation of fat is an autocatalytic reaction. The course of a typical autocatalytic oxidation of 4 g. of diced uncured bacon adipose tissue which was used in this study is shown in Figure 3. Although bacon adipose tissue contains only small amounts of heme compounds, these probably are the catalysts responsible for the rapid oxidation of bacon adipose. It should be pointed out, also, that the fat of the hog contains about 5% more dienoic fatty acids than does the fat of cattle *(1)* and thus oxidizes readily and makes pork products more susceptible to rancidtiy than beef products.

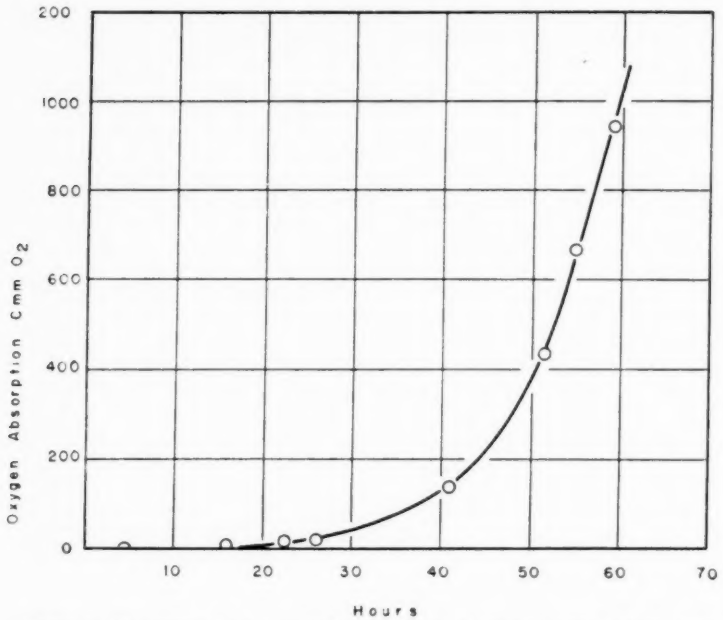

Figure 3. The course of the autocatalytic oxidation of uncured bacon adipose tissue at 10°C.

A study of the catalytic effect of crystalline hemin on the oxidation of linoleate shows that the catalysis conforms to the mathematical relationship:

$$-\frac{dO_2}{dt} = K\,(\text{Hemin})^{1/2}$$

Linoleate oxidation typically follows an analogous relationship when the reaction is initiated by linoleate peroxides or compounds like benzoyl peroxide *(3)*. Linoleate oxidations conforming to this relationship are believed to proceed by a chain reaction mechanism.

Because of the inhibitory effect of antioxidants on hemin catalyzed unsaturated fatty acid oxidation, Barron and Lyman *(2)* likewise concluded that hemin acts as a catalyst by promoting a chain reaction. The experimental results are presented graphically in Figure 4 where the initial reaction rate of oxygen absorption is correlated with the square root of the hemin concentration. The deviation from the linear relationship at high concentrations of hemin can best be explained by assuming that at high levels of hemin there is a displacement of linoleic acid molecules from the interface film and with the loss of heterogeneity a corresponding decrease in hemin catalysis *(10)*.

The catalytic mechanism of raw and cured bacon adipose extracts has been analyzed by this same technique. It was found that the oxidation of

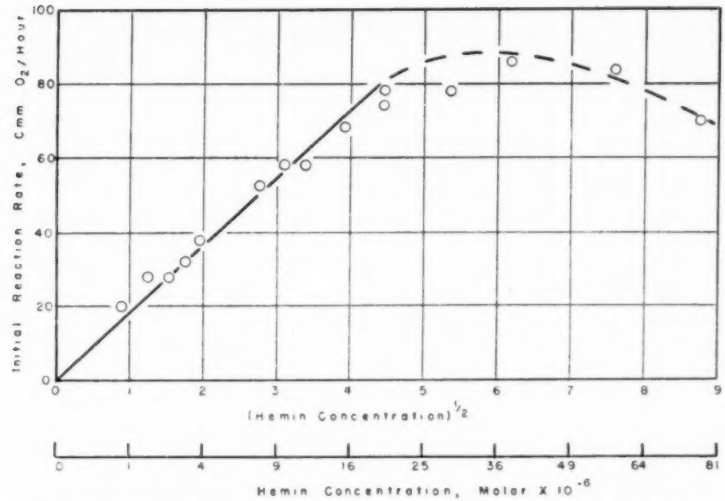

Figure 4. The initial rate of linoleate oxidation at 10°C. as a function of the concentration of crystalline hemin.

linoleate catalyzed by these 2 bacon adipose tissue extracts followed essentially the same mathematical relationship as did linoleate oxidation catalyzed by crystalline hemin. The experimental data presented in Figures 5 and 6 show that the concentration of catalyst present in the cured bacon adipose extract was much less than that present in the raw bacon adipose extract. This probably reflects the efficiency of separation of heme com-

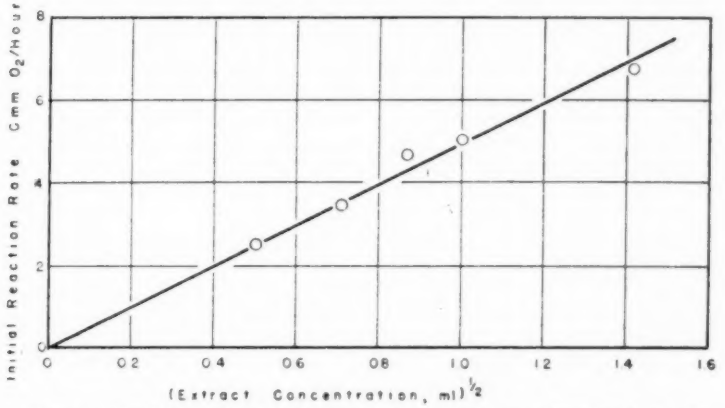

Figure 5. The initial rate of linoleate oxidation at 10°C. as a function of the concentration of cured bacon adipose extract.

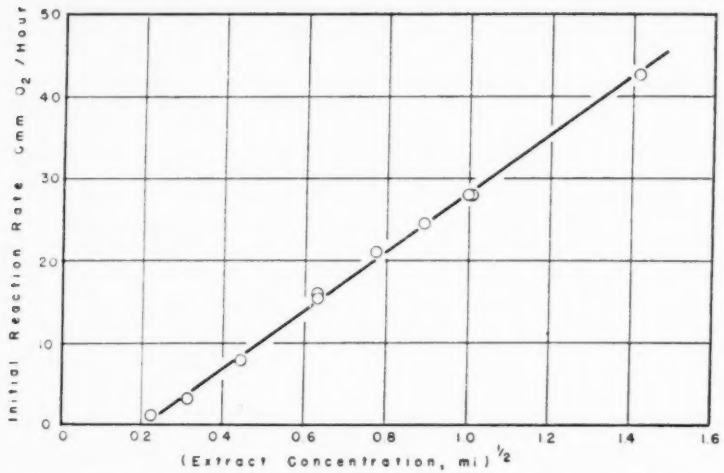

Figure 6. The initial rate of linoleate oxidation at 10°C. as a function of the concentration of uncured bacon adipose extract.

pounds out of the 2 different extracts. It is interesting that the straight line passing through the experimental points in the case of linoleate oxidation catalysis by raw bacon adipose extract does not intersect at the origin but rather at a positive value of extract concentration. Apparently there is an inhibitor present in this system which has the ability to inactivate a small portion of the active catalyst. Although the level of myoglobin and hemoglobin concentration in these bacon adipose extracts is too low to measure by the alkaline pyridine hemochromogen method, they are the only active catalysts known to occur in bacon adipose that could catalyze linoleate oxidation by a chain reaction mechanism at the high level noted in these experiments. Perhaps these observations offer an explanation for the catalysis by bacon adipose extracts which Reiser *(16)* found. If pork adipose extracts contain even very small amounts of heme compounds, these catalysts can function to initiate a chain reaction type of oxidation and their catalytic function may be greatly magnified.

SUMMARY

The catalysis of sodium linoleate oxidation by hog muscle and adipose tissue extracts has been studied.

The heat lability of linoleate oxidation catalysts in muscle extracts is more likely due to an effective removal of heme compounds by protein coagulation rather than a denaturation of enzymes which are capable of direct oxidation of linoleate.

By use of an assay method which eliminated interference of linoleate oxidation catalysis by heme compounds, the presence of any significant quantity of lipoxidase in pork tissues could not be demonstrated.

Bacon adipose tissue extracts promote a chain reaction oxidation of linoleate indicating that myoglobin and hemoglobin are the predominant catalysts involved.

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SPOILAGE OF CANNED FOODS CAUSED BY THE *BACILLUS MACERANS-POLYMYXA* GROUP OF BACTERIA

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Members of the *Bacillus macerans-polymyxa* group of bacteria have not been included among the major groups of canned food spoilage organisms even though Cameron, Esty, and Williams *(7)* proved a related species, *B. betanigrificans*,[c] to cause "black beets" and other mesophilic members of the genus are well known for their ability to spoil a variety of such foods. [See the reports of Crossley *(8)*, Fellers *(12)*, Gibson *(14)*, Jensen and Hess *(18)*, Jensen, Wood, and Jansen *(19)*, McMaster *(22)*, Michael and Tanner *(25)*, Nichols *(26)*, and Normington *(27)*.] Nevertheless, since 1943 several outbreaks of canned food spoilage have been observed in which it was possible to incriminate either *B. macerans* or *B. polymyxa* or a mixture of these 2 species as the cause of the spoilage. Gas formation, principally carbon dioxide but with some hydrogen, was the chief characteristic of all outbreaks. Sometimes tissue disintegration or polysaccharide (gum) formation was associated with the spoilage. Both tin and glass containers were involved in several outbreaks of spoilage observed in commercially canned fruits and vegetables and in home-canned fruits.

The first commercial outbreak called to the attention of the authors from which the facultative aerobic, gas-forming bacilli were isolated occurred in peas[d] packed in glass with a vacuum closure. Cooling water, heavily contaminated with the spores of the *B. macerans-polymyxa* group of bacteria, was "breathed" into the jars through a defective sealing surface (finish) which finally was closed by the rubber gasket before cooling was completed. In the second commercial outbreak of spoilage the same types of bacteria were recovered from fruits (cling peaches and a diced fruit mixture of cling peaches and pears) packed in tin cans. The exact cause of this second outbreak was not determined. The suspected bacteria were found in large numbers in the plant water supply and in the syrups used for packing the fruits as well as in the cooling water. Furthermore, the pH values of some of the cut-out syrups from sound cans were as high as 4.4, indicating excessive neutralization of the fruit which had been lye peeled. Consequently, opinion was divided as to whether cooling water contamination resulting from "breathing" or borderline processing was

[a] Present address: Curtis and Tompkins, Ltd., San Francisco, California.

[b] Present address: National Canners Association, Berkeley, California.

[c] This species is considered to be synonymous with *Bacillus macerans* by Smith, Gordon, and Clark *(32)*.

[d] The peas represented an out-of-state outbreak of spoilage not observed at first hand. W. E. Parks graciously supplied samples of spoiled peas as well as cultures which had been isolated at the source.

the cause of the trouble. During the same summer spoilage caused by these bacteria was observed in home-canned cling peaches and apricots, part of which were "cold-packed" in glass jars and part in tin cans. Later, additional representatives of this group of bacteria were recovered from spoiled commercially canned tomatoes, asparagus, and spinach. The lack of information concerning the possible rôle of *B. macerans* and *B. polymyxa* in canned food spoilage stimulated further study, the results of which are described in the following pages.

CHARACTERISTICS OF THE BACTERIA

Source of cultures. The cultures to be considered were isolated from different cannery waters, a canner's sucrose syrup for "choice" grade canned peaches, and a variety of spoiled canned fruits and vegetables including apricots, fruit cocktail, olives, cling peaches, diced mixed cling peaches and pears, asparagus, corn, peas, spinach, and tomatoes. The spoiled apricots and peaches included both commercial and home-canned samples. The peas represented a single glass-packed, commercial outbreak. The other samples had been packed in various sizes of tin cans in different canneries.

When collected or received for examination, the samples had undergone gassy fermentation in varying degree. The gas from the spoiled samples always contained appreciable quantities of hydrogen together with large amounts of carbon dioxide. However, the samples had no distinctive off-odor although they were always effervescent and, as already mentioned, sometimes the tissues were disintegrated and frequently the fruit syrups were gummy and ropy (see Figure 1).

Preparations made from spoiled samples and examined under the microscope were found to contain short and long rods. The latter frequently were extended into long filaments. No yeasts or cocci were observed. Attempts to recover viable cells or spores of members of the genus *Clostridium* were unsuccessful. Consequently, a search was made for other bacteria which might have produced the gas. Enrichment cultures of each sample were made in glucose, lactose, and sucrose broths incubated at 30°C.(86°F.). When gas was produced in these media, spore-forming, facultative aerobic, catalase-positive rods were recovered. When purified these cultures would reproduce the gassy spoilage when inoculated into the various foods from which they were recovered. As a result of these exploratory attempts all subsequent enrichments of spoiled samples were made in lactose broth as well as being inoculated into the conventional media used for examination of canned foods as described by Baumgartner *(3)* and Tanner *(33)*. The lactose medium could be successfully used because the bacteria, tentatively identified as aerobacilli, all fermented lactose. Twenty-three such cultures were selected for more detailed study.

Methods and media. The cultures, purified by serial replating on nutrient agar, were identified according to the methods suggested by Porter, McCleskey, and Levine *(30)* and Smith, Gordon, and Clark *(32)*. Primary differentiation of the cultures from other aerobic, sporeforming species of the genus *Bacillus* was made on the basis of differences in spore morphology and carbohydrate fermentation. Species identification was achieved through determination of the ability of the cultures to produce acetoin from glucose (positive V.-P. reaction); to produce crystalline dextrins from starch; to ferment sorbitol and rhamnose; and to grow in sucrose broth at 19°C.(66.2°F.) and 43.5°C.(110.3°F.).

The Voges-Proskauer reaction was determined on cultures grown in Difco M.R.-V.-P. medium at 30°C.(86°F.) for periods of from 1 to 5 days as recommended by Vaughn, Mitchell, and Levine *(37)*. The V.-P. reagent mixture described by Vaughn, Wedding, and Tabachnick *(38)* was added to 1 ml. of culture. The production of crystalline dextrins from starch was determined according to the method of Tilden and Hudson *(34)*. The ability to ferment sorbitol and rhamnose was tested in Durham tubes. These and other substrates, including sucrose, were added in concentrations of 1% to a basal medium which contained 5 g. of Bacto-peptone, 3 g. of Bacto beef extract, and 10 ml. of Andrade indicator per liter of distilled water.

The ability of the cultures to produce pectolytic enzymes was tested by growing them in the sodium ammonium pectate medium of Baier and Manchester *(2)* and on

Figure 1. Spoiled home canned peaches. Jar inverted to show gum formation.

sterile potato and carrot wedges. Methods used for determining the resistance of the cultures to acidity (pH), chlorine, heat, salt, and sugar will be considered separately.

Differentiation of the two species. All of the isolates were catalase-positive, sporulating, gas-forming, facultative aerobic, gram variable rods of the genus *Bacillus* with general characteristics which identified them with the *B. polymyxa-B. macerans* group as described in detail by Porter, McCleskey, and Levine *(30)* and Smith, Gordon, and Clark *(32)*. The cultures were particularly characteristic of this group in that they all conformed to the previously described limits of cell and spore morphology and all fermented carbohydrates with the production of acid and gas. Differential characteristics which permitted allocation of the bacteria to the 2 species are shown in Table 1. The species *B. polymyxa* was represented by 18 strains; the species *B. macerans* by

TABLE 1

Differential reactions of the bacteria

Differential characters	Species allocation	
	B. polymyxa	*B. macerans*
	18 cultures	5 cultures
	Number of cultures giving positive reactions	
Voges-Proskauer reaction	18	0
Production of crystalline dextrins from starch[e]	0	5
Gas production in sucrose broth at 19°C.(66.2°F.)	18	0
Gas production in sucrose broth at 43.5°C.(110.3°F.)	0	5
Gas production from rhamnose[e]	5[f]	5
Gas production from sorbitol[e]	5[f]	2

[e] Incubated at 37°C.(98.6°F.).
[f] The same 5 cultures fermented both substrates.

5 isolates. On the basis of the results of the Voges-Proskauer reaction, the test for the production of crystalline dextrins from starch, and production of gas in sucrose broth incubated at 19°C.(66.2°F.) and 43.5°C.(110.3°F.), there was perfect agreement with the previously described characteristics which have been used to separate the 2 species.

However, as can be seen from the table, the results of the fermentations of rhamnose and sorbitol were not clear cut. Porter, McCleskey, and Levine *(30)* reported that *B. macerans* fermented both rhamnose and sorbitol with the production of acid and gas whereas *B. polymyxa* did not attack either substrate. Tilden and Hudson *(34)* also found that their cultures of *B. polymyxa* did not ferment rhamnose. On the other hand, Smith, Gordon, and Clark *(32)* found that all of their cultures of *B. polymyxa* fermented both compounds and, after additional experiments with rhamnose, concluded that the fermentation of rhamnose and sorbitol was a variable character. The results obtained in this investigation indicate that 2 types of *B. polymyxa* exist: one (represented by 13 cultures) ferments neither rhamnose nor sorbitol; the other (represented by 5 cultures) attacks both compounds. The variations in the ability of these 2 species to ferment these 2 compounds is also emphasized by the failure of 3 of the 5 cultures of *B. macerans* to ferment sorbitol. Other fermentable carbohydrates appeared to offer no better means of differentiation as all those tested were attacked by other species. Gum formation from sugars by the species *B. polymyxa* also was found to be too variable to be of value as a differential test.

Pectolytic activity. Ability to cause fermentation and disintegration of carrot and potato wedges was possessed by all cultures of both species. However, a marked difference in pectolytic activity was noted when the cultures were grown on the sodium ammonium pectate medium of Baier and Manchester *(2)*. All of the cultures of *B. polymyxa* completely liquefied the medium within 2 weeks whereas the cultures of *B. macerans* caused only slight softening during the same period. The marked difference noted in destruction of sodium ammonium pectate gel by these 2 species might possibly aid in their further differentiation.

Salt tolerance. A number of the cultures were isolated from canned vegetables and olives whose brines contained varying concentrations of salt. Since information concerning the salt tolerance of *B. polymyxa* and *B. macerans* was not found in the available literature, the resistance of the cultures to salt was investigated.

Durham tubes of sucrose nutrient broth with known concentrations of salt (grams per 100 ml. determined by silver nitrate titration) were used for the salt tolerance studies. The inoculum consisted of a 4-mm. loopful of an active 24-hour nutrient broth culture. The inoculated tubes were incubated at 37°C.(98.6°F.) and observed for growth, acid, and gas production daily for 2 weeks. The cultures were tested with and without acclimatization according to the method described by Foda and Vaughn *(13)*.

All *B. polymyxa* cultures grew vigorously in the presence of 1 and 2% salt. Growth of 9 of the 18 strains investigated was inhibited by 4% salt. The remaining 9 unacclimatized isolates were inhibited by 6% salt. All of the untrained *B. macerans* cultures grew well in the presence of 1 and 2% salt but were completely inhibited when the concentration was increased to 4%.

On acclimatization 4 of the 18 *B. polymyxa* cultures were trained to tolerate 7% salt. Three of these 4 cultures could grow in the presence of 8% salt, but all failed to grow when the concentration was increased to 9%. The *B. macerans* cultures were trained to grow in the 4% salt medium but all were inhibited when the concentration was increased to 5%. It is obvious therefore that the concentrations of salt used in brines for canning of vegetables and ripe olives would not prevent the growth of these bacteria.

Sucrose tolerance. The possibility that the sucrose syrups used for the preparation of choice or fancy grade canned fruits would prevent growth of the aerobacilli suggested an investigation of their tolerance to sugar.

Durham tubes of sucrose nutrient broth containing from 10 to 60% sugar by weight were prepared and sterilized by heating in live steam, 100°C.(212°F.), for 1 hour on 3 successive days. The tubes containing various concentrations of sugar then were inoculated with a 4-mm. loopful of an actively growing 24-hour nutrient broth culture and incubated at 37°C.(98.6°F.) for 3 weeks. Daily observations for acid and gas production were made.

All but one of the 18 *B. polymyxa* cultures grew well in the presence of 30% sucrose and 5 strains grew, though less actively, at a concentration of 35%. Two of the cultures grew when the sucrose was increased to 40% but failed to grow when the concentration was raised to 45%. All *B. macerans* cultures grew in the presence of 25% sugar but none could grow in higher concentrations. No attempt was made to train the cultures of either species to tolerate more sugar.

B. polymyxa strains are able to tolerate more sugar than the *B. macerans* cultures. It is also evident that choice to fancy sirups, *i.e.* those ranging from 40 to 55° Balling, would eliminate the aerobacilli as potential agents of spoilage of the syrups themselves. However, since the "cut-out" concentrations of these syrups would be likely to fall below the inhibitory concentrations for the 2 species, spoilage—although retarded—might occur in canned fruits.

Chlorine resistance. Cameron *(6)*, Merrill *(23)*, Scott *(31)*, and Woerz *(41)* have shown that one of the principal sources of potential contamination of canned foods is the cooling water, and that with reduction of the bacterial contamination of the water by chlorination, spoilage of canned foods attributed to leakage contamination can be substantially reduced. Water has been shown to be an important source of the aerobacilli *(5, 9, 11, 15, 16, 17, 20, 24, 28, 30, 39)* and, as already mentioned, some of the cultures included in this investigation were isolated from samples of cooling water. Information concerning the chlorine resistance of these bacteria was not found in the literature. Consequently, it was desirable to determine the resistance of the spores of representative cultures of *B. polymyxa* and *B. macerans*.

The tests were made with spore suspensions prepared according to the method of Magoon *(21)*, adjusted to contain 5,000,000 spores per ml., and stored at 4°C.(39.2°F.) when not in use. Solutions containing 5, 10, 25, 50, 75, and 100 p.p.m. of chlorine, were made in chlorine demand-free distilled water by addition of calcium hypochlorite solution. Available chlorine was determined by the acidic iodometric titration method *(1)*. The test solutions, adjusted to 20°C.(68°F.) and maintained at this temperature for the duration of the run, were prepared so that 100 ml. contained the desired concentration of chlorine and 50,000 spores per ml. at zero time. Fresh solutions were prepared for each run.

The spores were exposed to the different concentrations of chlorine for periods ranging from 5 to 300 minutes. After exposure a 1-ml. portion was withdrawn from each test sample and the chlorine neutralized with 1 ml. of thiosulfate solution. This combined portion then was inoculated into 0.5% sucrose nutrient broth which contained 0.5% soluble starch. The work of Olsen and Scott *(29)* had shown that the presence of soluble starch facilitated germination of heat-treated spores. It appeared that the presence of soluble starch also aided germination of spores subjected to chlorine because, after its incorporation into the recovery medium, "skips" were reduced to a minimum. Acid and gas production in the sub-culture tubes were used as the criteria for survival of the spores. The subculture tubes were incubated at 37°C.(98.6°F.) for 2 weeks.

The results of a typical run are shown in Table 2. The data indicate that the spores of *B. macerans* may be slightly more resistant to chlorine than those of *B. polymyxa*. However, it is to be stressed that the limited collection of 5 cultures is not likely to

TABLE 2

Effect of chlorine on washed spores[a] of B. polymyxa and B. macerans

Culture	Chlorine	pH	Exposure time in minutes at 20°C.(68°F.)									
			5	10	15	20	25	120	210	220	240	270	300
	p.p.m.												
	100	10.84	+	+[b]	—[t]	—	—	—	—	—	—	—	—
	75	10.64	+	+	+	—	—	—	—	—	—	—	—
B. polymyxa	50	10.52	+	+	+	+	—	—	—	—	—	—	—
No. 18	25	10.25	+	+	+	+	+	+	—	—	—	—	—
	10	9.95	+	+	+	+	+	+	+	—	—	—	—
	5	9.52	+	+	+	+	+	+	+	+	—	—	—
	100	10.84	+	—	—	—	—	—	—	—	—	—	—
	75	10.64	+	+	—	—	—	—	—	—	—	—	—
B. macerans	50	10.52	+	+	+	+	+	—	—	—	—	—	—
No. 5	25	10.25	+	+	+	+	+	+	+	+	—	—	—
	10	9.95	+	+	+	+	+	+	+	+	+	—	—
	5	9.52	+	+	+	+	+	+	+	+	+	+	—

[a] — 50,000 spores per ml. of reaction mixture.
[b] + indicates survival and germination of spores in subculture medium.
[t] — indicates destruction of spores.

represent the limits of resistance to chlorine of the various types of *B. macerans*. It may be that more resistant forms of *B. polymyxa* also may be found.

pH tolerance. Vaughn and Stadtman *(36)* reported that cultures of *B. macerans* were capable of causing spoilage of canned fruits having syrups with pH values of 3.8 to 4.0.

In the present study it was found that the limiting pH for growth of cultures of either *B. polymyxa* or *B. macerans* was in the range of 3.8 to 4.0 when the cultures were grown in sucrose nutrient broth or 20° Balling syrup from canned cling peaches. It was not possible, in the time available, to train cultures of either species to tolerate lower pH values despite the fact that some of the spoiled peaches investigated had syrups with pH values of 3.6 as determined by the glass electrode.

Heat resistance. As already stressed, it was not always possible to state explicitly whether borderline processing or "breather" contamination was the cause of the outbreaks of spoilage. However, even though the spores of the aerobacilli were destroyed by prescribed processing temperatures, cans of food still might become infected through postprocessing leakage of contaminated cooling water and spore germination and vegetative multiplication would result in spoilage. Therefore it was desirable to investigate the heat resistance of the spores of representative cultures of *B. polymyxa* and *B. macerans*.

From the literature the impression was gained that the aerobacilli might have considerable heat resistance. Zacharov *(42)* reported that the spores of *B. macerans* were able to resist 100°C.(212°F.) for 3 or more hours. Cameron, Esty, and Williams *(7)* also believed that the original thermal resistance of the spores of the related species, *B. betanigrificans*, "*must have been materially higher than was indicated by the laboratory tests.*"

The spore suspensions were prepared in sand by the method of Magoon *(21)* as adapted to the present study. The sand used was ordinary, clean, 40-mesh sea sand which was heated with dilute hydrochloric acid for 10 minutes, neutralized with dilute sodium hydroxide, washed repeatedly with tap water, and finally with distilled water before drying in the hot-air sterilizing oven. Then 25-g. portions of the dry sand were evenly distributed in Petri dishes and sterilized in dry air at 160°C.(320°F.) for about 12 hours before use.

The spore preparations were obtained from cultures grown on nutrient agar slants (pH 6.8) held at room temperature for 7 days. The growth from each slant was suspended in 7 ml. of nutrient broth (pH 7.0) which had been added aseptically to the culture. The suspensions were transferred to sterile pyrex tubes and heated in a water bath at 80°C.(176°F.) for 10 minutes to kill the vegetative cells. The heated suspen-

sions then were distributed dropwise, by means of a sterile pipette, over the surface of the sterile sand culture plate until the sand was saturated. The sand-spore mixture then was incubated at 30°C.(86°F.) for 10 days.

After incubation about 3 g. of the sand culture was removed by means of a sterile spatula and transferred to 100 ml. of sterile distilled water contained in a screw-cap bottle. Vigorous shaking by hand served to free the spores from the sand, break up any clumps, and suspend the individual spores in the water. The clear supernatant then was pipetted in 25-ml. portions into sterile 50-ml. centrifuge tubes. The spores were separated by centrifugation at high speed. The supernatant was removed by sterile pipette and the spores resuspended in 25 ml. of sterile distilled water. After centrifugation, and washing once more in 25 ml. of sterile distilled water, the spores were separated again and suspended in 2 ml. of sterile distilled water. This washed spore suspension was standardized by means of the Neubauer counting chamber and used immediately or stored at 0°C.(32°F.) until needed. Fresh spore suspensions were prepared for each series of tests. Four different media were used for testing the thermal resistance of the spores. These included M/15 Sørensen's phosphate buffer at pH 7.0; canned spinach liquor at pH 6.8; canned spinach liquor at pH 4.8; and 16.5° Balling syrup from canned, diced, and mixed peaches and pears at pH 3.6. The spores were used in a final concentration of 50,000 per ml. in each of these suspending media.

To insure uniformity the spinach liquors and the fruit syrup were prepared from cans of each product which bore the same brand labels and code markings. Great care was taken to prepare the liquors and syrup under aseptic conditions in order to reduce the time necessary to sterilize them without undue heat damage. By such procedure it was possible to sterilize the spinach liquors in 10 minutes at 15 p.s.i.g. and the fruit syrup in 10 minutes at 2 p.s.i.g. The pH of the spinach liquor was adjusted to 6.8 with sodium hydroxide. All pH determinations were made with the glass electrode on sterilized, cooled samples.

Heat resistance was determined by the sealed tube method first described by Bigelow and Esty *(4)* and later modified by Esty and Williams *(10)*, Williams *(40)*, and others. The desired spore suspension was dispensed in 2-ml. portions into pyrex tubes 10 cm. long with an inside diameter of 7 mm. and a wall thickness of 1 mm. The tubes were sealed and submerged in the oil bath at the desired temperature as soon after filling as possible. A lag period of 1.5 minutes was allowed for the tubes to come to the temperature of the bath before the start of heat exposure was recorded. Three temperatures, 90, 95, and 100°C.(194, 203, and 212°F.) were used throughout the investigation. At intervals of 1 or 2 minutes, pairs of the tubes were removed from the bath and immediately cooled in ice water. The 1-minute interval was used when the exposure time approached the critical point between survival and destruction of the spores.

In these tests it was found advantageous to nick the heated tube with a file at least one-half inch above the level of the contents and remove the tip with a pair of flamed pliers. Then 1 ml. of the contents was removed with a sterile pipette whose ungraduated extremity had been blown to a long, narrow tip. These procedures materially aided in prevention of possible contamination of the heated suspension on transfer to the recovery medium.

The subculture medium was composed of 5 g. of Bacto-peptone, 3 g. of Bacto-beef extract, 5 g. of sucrose and 5 g. of soluble starch per liter of distilled water. The pH was adjusted so that 9 ml. of sterile medium mixed with 1 ml. of heated spore suspension was in the range of 6.8 to 7.0. The adjustment of the pH of the recovery medium and the use of soluble starch as recommended by Olsen and Scott *(29)* materially aided and hastened spore germination and all but eliminated "skips" in the tests made.

The inoculated subculture tubes were incubated at 37°C.(98.6°F.). Observations of growth, acid, and gas production were made daily for the first week and at weekly intervals thereafter until the tubes were discarded. However, the results observed in the first 4 days did not change despite prolonged incubation. The results were recorded in terms of survival and destruction times. The exposure time of the last tubes showing growth was taken as the survival time at that temperature. The results are all based on at least 2 determinations and some on as many as 4 determinations at the same temperature with the same test medium. The results are presented graphically as thermal death time curves for spores of the 2 species in Figures 2 and 3.

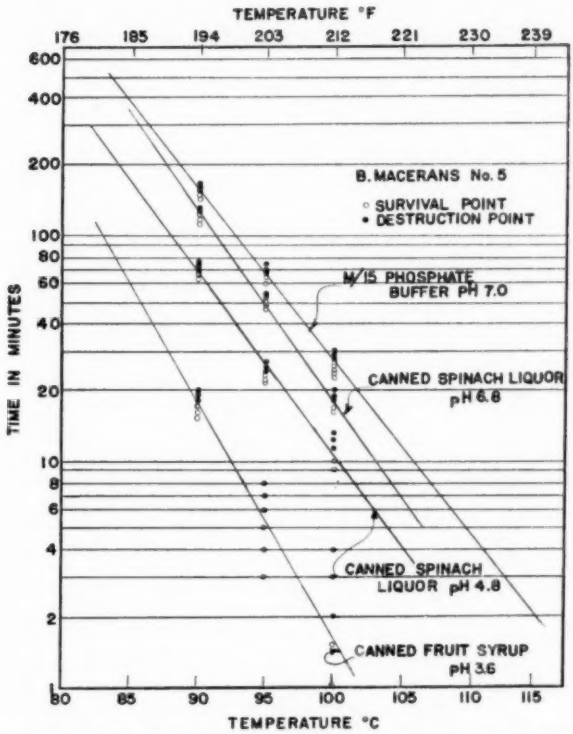

Figure 2. Thermal death time curves for spores of B. macerans, strain No. 5.

It is evident on comparison of these thermal death time curves that the spores of *B. macerans*, strain No. 5 (Figure 2), possess slightly more thermal resistance than those of *B. polymyxa*, strain No. 18 (Figure 3). It is also obvious that the resistance of the spores of either species to heat is not particularly high but of about the same magnitude as that shown for the spores of *Clostridium pasteurianum* by Townsend *(35)*. The extreme thermal resistance of the spores of *B. macerans* reported by Zacharov *(42)* could be duplicated by the authors only when the spores were heated in cotton-plugged tubes or flasks.

DISCUSSION

Evidence accumulated during the course of this investigation suggested that 2 of the outbreaks of spoilage, whose histories were known in sufficient detail, probably were caused by contaminated cooling water breathed into the cans of product during post-process cooling.

With the exception of the glass-packed peas and home-prepared products, all of the canned foods investigated had been processed in continuous, rotary, pressure cookers and coolers. Evidence of gross leakage could not be demonstrated in normal or spoiled cans of food processed during the same operating period. Furthermore, the normal cans were found to be

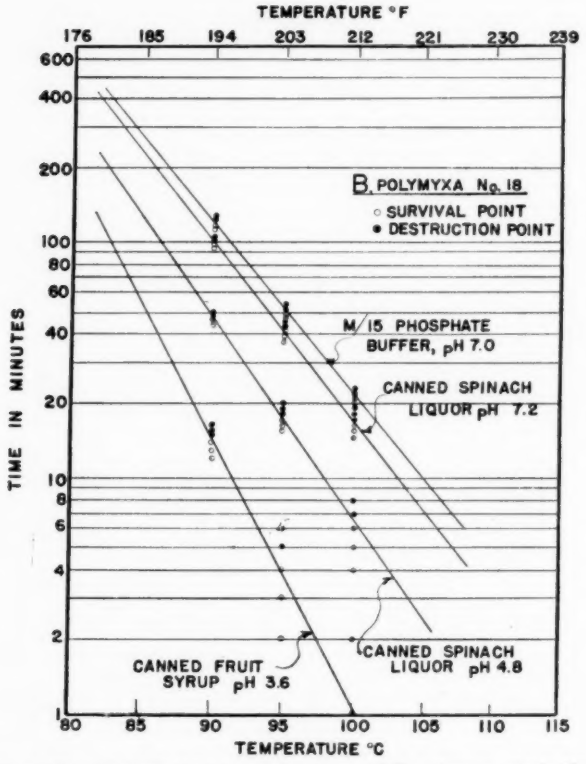

Figure 3. Thermal death time curves for spores of B. polymyxa, strain No. 18.

well within the tolerance suggested by the manufacturers for the types of cans used and all contained vacuums within the recommended ranges. Also, as nearly as could be determined, the processing temperatures and times were in conformance with recommendations, and increases in processing temperatures and times did not eliminate the spoilage. However, in 1 of the 2 cases, even though operation of the continuous pressure cooker-cooler called for an over-riding pressure of air, there was none. The cooling waters were found to be contaminated with *B. macerans* or *B. polymyxa* or mixtures of the 2 species. Therefore, circumstantial evidence pointed to "breather" spoilage because the outbreaks in question were eliminated when the cooling waters were chlorinated sufficiently to eliminate the aerobacilli and the cookers were operated correctly.

There are several reasons for incrimination of the aerobacilli in breather spoilage. The 2 species commonly are water-borne. Because they grow at temperatures of from 40°C.(104°F.) to 50°C.(122°F.), they might actually multiply in compartmentized rotary coolers. Furthermore,

spores of the 2 species have sufficient heat resistance to withstand the temperatures found in rotary coolers even though their vegetative cells might be killed.

Application of more thorough sanitary practices including strict control of the cooling water supplies, installation of in-plant, break-point chlorination using 2 to 5 p.p.m. of chlorine in the cooling waters, and more efficient operation of the processing equipment were combined to control these bacteria under operating conditions in the canneries.

SUMMARY

A group of facultative aerobic, spore-forming aerobacilli related to or identical with *B. polymyxa* and *B. macerans* were described. The cultures were found to be of importance in the gaseous deterioration of canned foods because they were quite tolerant to acidic environments and to reasonably high concentrations of sucrose. Furthermore, their spores were fairly resistant to chlorine and to heat.

Effective control of the bacteria under practical operating conditions was possible by application of more thorough sanitary practices including strict control of the cooling waters, use of break-point chlorination with 2 to 5 p.p.m. of chlorine in the cooling waters, and more efficient operation of the processing equipment.

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EFFECTS OF HIGH INTENSITY ELECTRON BURSTS UPON VARIOUS VEGETABLE AND FISH OILS*

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The pronounced efficiency of penetrating, ionizing radiation in the inactivation of microorganisms is by now a well-established fact *(1, 3, 19, 20)*. Coupled with it is a remarkable prolongation of storage time of organic matter, often without significant structural alterations *(1, 9)*.

The heat equivalent *(10)* of a sterilizing dose of high intensity electrons is small, i.e. about 3.7 cal. per g. and Traub, Huber, and Brasch *(24)* have furthermore shown that the killing efficiency with high intensity electron bursts of many microorganisms is substantially independent of temperature in the range of 20°C. to —180°C. One can therefore describe the radiation inactivation of microorganisms as "cold sterilization" *(2, 21)* and the potential practical importance of such a technique in the preservation of perishables is readily apparent.

It is of considerable importance to establish whether or not irradiated products, destined for human consumption, undergo detrimental changes with doses of electrons sufficient to achieve complete inactivation of bacteria and spores, and a broad program in this direction has been under way in this laboratory for the last 4 years.

In conjunction with work on the radiation sterilization of certain oil-bearing vegetative products and a variety of fish, we have investigated the effect of sterilizing doses of high intensity electron bursts on cottonseed, castor, and linseed oil as well as on cod liver, herring, mackerel, and whale oil. A series of chemical tests together with organoleptic evaluations were used to determine the type and the magnitude of changes that are taking place in the oils, due to the irradiation.

Few data are available on the effect of large doses of ionizing radiation upon oils and fats. Whitehead, Goodman, Breger *(25)* investigated the decomposition of fatty acids by alpha particles and observed decarboxylation as well as dehydrogenation, the rate being independent of temperatures below 0°C. Nickerson, Goldblith, and Proctor *(18)* investigating the chemical changes in mackerel tissues treated with electrons obtained from an electrostatic generator, observed accumulation of volatile acids in the tissues which they described as caused by the action of lipases upon the oils present in the tissues. Recently Hannan and Boag *(7)* studied the effect of electron irradiation on butter fat and observed the development of characteristic oxidative changes, with the apparent peroxide values depending on the temperature of the sample during irradiation. Contrary to the usually observed reduction of chemical effects on irradiation at low temperatures, these authors found that the peroxide values increased considerably with decrease of temperature.

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EXPERIMENTAL

The vegetable oils were purchased in the open market and were of commercial quality. The fish oils were obtained through the courtesy of Dr. E. Heen, Fisheries Research Institute, Bergen, Norway, from a commercial Norwegian source where they had been freshly prepared just prior to shipment. The mackerel oil was prepared directly from fresh, macerated fish muscle by repeated chloroform extraction in the presence of anhydrous sodium sulfate. The mixture was shaken mechanically for periods of 30 minutes until all the oil had been extracted. The chloroform extracts were combined and dried over fresh anhydrous sodium sulfate and the oil was then obtained by solvent removal in vacuum at room temperature. Mackerel oil prepared in this manner consistently had very low peroxide values.

The irradiation was carried out with high intensity electron bursts obtained from a 3MV Capacitron. The electrons are emitted from the generating tube into the air in bursts of about one microsecond duration with each burst containing an energy equivalent to hundreds of millions of rep gm. Rep *(3)* equals Roentgen Equivalent Physical and is a unit which represents the amount of energy which is absorbed in each gram of irradiated foodstuff. The equipment as well as the technique of irradiation and the dosage calculation have been thoroughly described elsewhere *(10, 11, 22)*.

For exposure the samples were packed either in specially blown glass ampules, 55 × 35 mm.; or in aluminum cans, 12 × 78 mm.; or in tin cans, 18 × 65 mm. The ampules were heat-sealed and the cans closed mechanically by an appropriate closing machine. The amount of irradiated sample varied from 7 g. in the ampules to 30 g. in the aluminum cans.

The degree of unsaturation was determined by iodine number using the Wijs *(14)* method.

The rancidity formation was followed by peroxide values according to the method of Lea *(15)*, using a mixture of chloroform and glacial acetic acid as solvent.

Changes in molecular aggregation were followed by viscosimetry using a standard Ostwald-Fenske type pipette.

Free fatty acids were determined by titration in hot 95% alcohol using the method of Williams *(26)*.

The formation of lower aldehydes was followed by the color formation in an etheral solution of phloroglucinol according to Kreis *(13)*.

RESULTS

The effects of a sterilizing dose of high intensity electron bursts—e.g. 1.5×10^6 rep on castor oil, cottonseed oil, as well as raw and boiled linseed oil at room temperature and 115°C.—are summarized in Table 1. In general it can be seen that the effect of such a dose of radiation causes, if any, only minor changes in the vegetable

TABLE 1

Effect of high intensity electrons on vegetable oils
Dose: 1.5×10^6 rep.

	Iodine number, g. I_2/100 g. oil		Kreis test, optical density		Peroxide value, μ mol./g. oil		Viscosity centipoise, 25°C.		Acid value, mg. KOH/g. oil	
	Irr.	Control	Irr.	Control	Irr.	Control	Irr.	Control	Irr.	Control
Castor oil										
20°C.	81.0	89.7	0.11	0.13	0.3	0	721.0	692.0	0.4	0.4
115°C.+2.5% $ZnCl_2$ anhyd	83.3	83.3	.....	.....	....	....	749.0	647.0	15.5	11.6
Cottonesed oil										
20°C.	112.9	111.0	0.10	0.11	0.8	0.2	55.9	55.9	2.7	2.7
115°C.+2.5% $ZnCl_2$ anhyd	107.8	110.2	.....	.....	....	....	55.8	54.8	3.5	2.6
Boiled linseed oil										
20°C.	178.2	189.7	0.26	0.15	4.0	1.0	55.7	49.0	6.3	6.0
115°C.+2.5% $ZnCl_2$ anhyd	171.1	176.2	.....	.....	....	....	44.7	44.5	6.1	5.4
Raw linseed oil										
20°C.	171.2	177.4	0.13	0.13	2.6	2.1	55.0	49.0	6.4	5.8

oils. Irradiation at 115°C. and in the presence of 2.5% anhydrous zinc chloride does not seem to instigate changes where there have been none at room temperature, nor to increase the magnitude of the changes occurring at room temperature. Changes due to irradiation were observed in castor oil and linseed oil. In castor oil, the iodine number decreases by about 10%, with a corresponding increase in viscosity, while Kreis test, peroxide value and acid value are essentially unchanged against the untreated control. In linseed oil there is also about a 10% drop in the iodine number and a corresponding increase in viscosity. This, however, is accompanied by a small but consistent increase in peroxide value, Kreis test, and acid value, indicating a more complex reaction.

Organoleptic changes are summarized in Table 2. They were obtained by a 5-member taste panel. Changes due to the irradiation were generally found to be not very pronounced. The odor of irradiated, raw linseed oil was slightly fishy, whereas the odor of cottonseed and castor oil had acquired a slightly sweet, ketonic quality. The taste of raw and boiled linseed oil was unchanged due to irradiation with 1.5×10^6 rep. In cottonseed oil a slightly more smooth taste was developed while in castor oil a more metallic quality became apparent. To evaluate the order of magnitude of these changes, the irradiated oils were diluted with equal volumes of untreated oil until the panel could not detect any difference between irradiated and control oil. Both in cottonseed and castor oil this ratio was 1 to 4.

Exploratory experiments with electron-sterilized fish oils indicated that the most pronounced change was in the development of rancidity during storage subsequent to the irradiation. This method therefore was used for the evaluation of the magnitude of such changes, particularly since other properties were but slightly altered. This is demonstrated by Table 3 which shows that the acceleration of rancidity formation by radiation does not seem to be accompanied by liberation of free fatty acids since the acid values in the tested oils remain unchanged over storage periods of up to 52 days at 25°C. irrespective of the presence or absence of an antioxidant.

Figure 1 shows the development of rancidity in whale oil stored at 25°C. after irradiation with 1.5 million rep. It can be seen that the rancidity formation in the irradiated oil proceeds more rapidly than in the untreated control with the value of the irradiated samples being about 80% higher after 2 weeks of storage.

Since it had previously been shown *(1)* that in many cases sterilization at low temperature was beneficial in the elimination of nondesirable chemical side-effects, the whale oil was also irradiated at −35°C. The corresponding curve in Figure 1, however, demonstrates that rancidity formation is not retarded by irradiation at low temperatures. Figure 2 shows a similar pattern for the development of rancidity in fresh mackerel oil. In this instance the storage time had been increased to 52 days at 25°C. and it can be seen that the rancidity formation in the irradiated oil slows down after an initial steep increase and that eventually the values of irradiated and control samples seem to converge toward the same values. Again, irradiation at −35°C. does not seem to decrease the rate of peroxide formation.

Since the development of rancidity in the irradiated oils seemed to depend upon the accelerated interaction of radiation-activated oxygen with unsaturated bonds, we attempted to eliminate the primary cause, e.g. oxygen or air, by irradiation in vacuum or under nitrogen as well as by the addition of antioxidants prior to the irradiation. The appropriate curves in Figures 1 and 2 demonstrate that both in the case of whale oil and mackerel oil, there is no noticeable increase in peroxide values, if the irradiation is carried out in vacuum or under nitrogen. The irradiations, in these cases, were carried out at room temperature and the samples were stored at 25°C. The reason why the nitrogen curve is consistently slightly above the vacuum curve is probably explained by the fact that the gas contains small amounts of oxygen of the order of about 1%, which in turn can be activated by the radiation, thus facilitating peroxide formation up to the extent of its initial concentration.

It is of interest that in all cases of irradiation in vacuum or nitrogen there seems to be a slight initial drop in peroxide values directly after irradiation; these revert back towards the starting values after several days of storage. The drop is particularly pronounced in mackerel oil and is demonstrated by insert A in Figure 2. The initial rate of peroxide formation in mackerel oil irradiated with high intensity electron bursts, is further demonstrated by Table 4. The results indicate that within one-half

TABLE 2

Organoleptic evaluation of vegetable and fish oils irradiated in air
Dose: 1.5×10^6 rep.

	Plain		Antioxidant added		Remarks
	Irradiated	Control	Irradiated	Control	
Raw herring oil					
Odor	Bland, sl. fishy	Fishy	N. ch.[a]	N. ch.[f]	N. ch. after 6 weeks' storage at 25°C.
Taste	Mild	Raw	N. ch.	N. ch.	
Refined herring oil					
Odor	Bland	Sl. fishy	Fishy	N. ch.[g, h]	N. ch. after 6 weeks' storage at 25°C.
Taste	Milder	Fishy, raw	Raw, bitter	Sl. bitter	
Raw cod liver oil					
Odor	Fishy	Sl. fishy	Fishy	N. ch.[g]	N. ch. after 6 weeks' storage at 25°C.
Taste	Sl. raw	Bland	Raw, bitter	Sl. bitter	
Refined cod liver oil					
Odor	Sl. fishy	Bland	N. ch.	N. ch.[i]	N. ch. after 6 weeks' storage at 25°C.
Taste	Sl. fishy	Bland	N. ch.	N. ch.	
Raw whale oil					
Odor	More neutral	Fishy	N. ch.	N. ch.[j]	N. ch. after 6 weeks' storage at 25°C.
Taste	More fishy, burnt	V. fishy and acrid	Increasingly burnt	N. ch.	
Refined whale oil					
Odor	Sl. fishy	Neutral	.....	.....	Taste and odor revert back to control value after 6 weeks' storage at 25°C.
Taste	Fishy	Bland, sl. burnt	.....	.....	
Raw linseed oil					
Odor	Fishy	Moldy hay	.....	.....	Flavor value:[k] 1
Taste	Bitter, stronger than control	Fishy	.....	.....	
Boiled linseed oil					
Odor	Milder	Burnt, fishy	.....	.....	Flavor value: 1
Taste	Milder, sweetish	Strongly fishy	.....	.....	
Cottonseed oil					
Odor	Sweet hay, ketonic	Bland	.....	.....	Flavor value: 4
Taste	N. ch.	Bland	.....	.....	
Castor oil					
Odor	Flowery, ketonic	Bland	.....	.....	Flavor value: 4
Taste	Burnt, metallic	Sharp, bitter	.....	.....	

[a] No change. [f] 0.05% tocopherol. [g] 0.05% propylgallate. [h] 0.1% butylated hydroxyanisole. [i] 0.1% palmitoyl ascorbic acid. [j] 0.05% ascorbic acid.
[k] × dilution with equal volume of untreated oil to make irradiated sample indistinguishable from control.

TABLE 3

Free fatty acids in irradiated fish oils
Dose: 1.5 × 10^6 rep.

	Acid values (mg. KOH/g. oil) at various storage times (days)		
	0	13	52
Raw whale oil			
Control	2.6	2.6	2.6
Irradiated	2.6	2.6	2.6
Refined whale oil			
Control	0.4	0.4	0.4
Irradiated	0.4	0.4	0.4
Raw cod liver oil			
Control plain	7.3	7.3	7.3
Control—0.05% propylgallate	8.1	7.8	7.8
Irradiated plain	7.3	7.3	7.3
Irradiated—0.05% propylgallate	8.1	7.8	7.8
Refined cod liver oil			
Control plain	1.2	1.2	1.2
Control—0.05% propylgallate	1.4	1.3	1.4
Irradiated plain	1.3	1.3	1.2
Irradiated—0.05% propylgallate	1.4	1.3	1.4

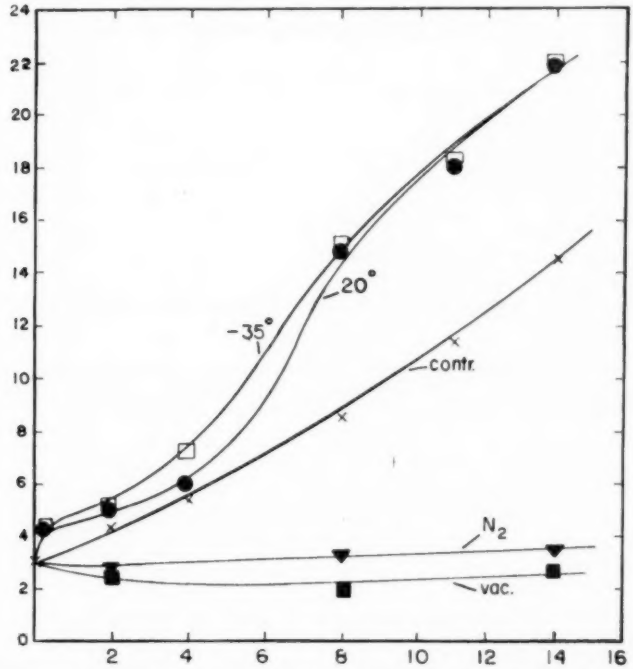

Figure 1. Development of rancidity in refined whale oil irradiated under various conditions. Dose: 1.5 × 10^6 rep. Irradiated and stored at 25°C. Abscissa, time in days after irradiation. Ordinate, peroxide value in μ mols per gram of oil.

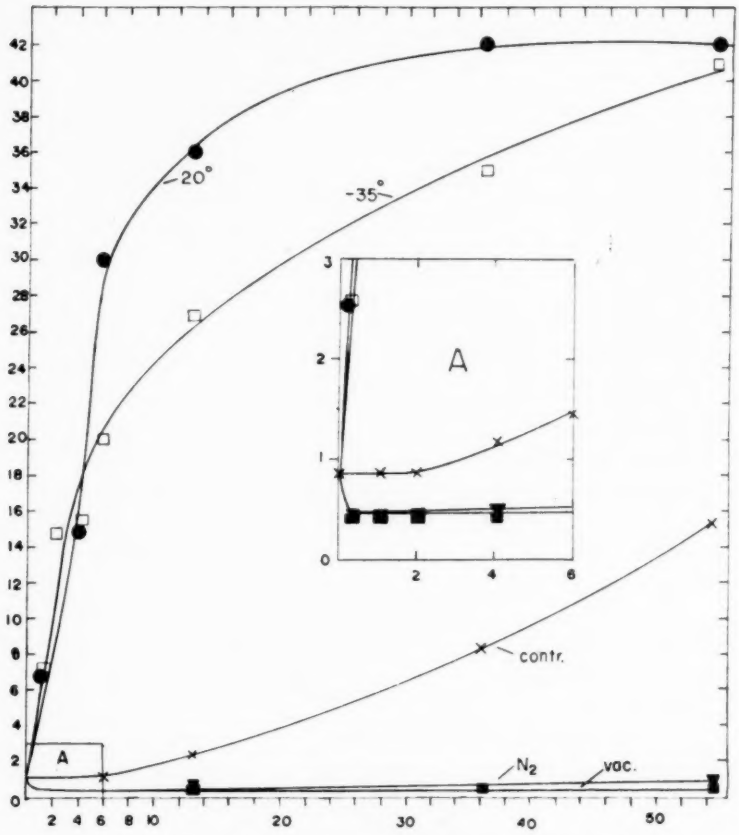

Figure 2. Development of rancidity in fresh mackerel oil irradiated under various conditions. Dose: 1.5×10^6 rep. Irradiated and stored at 25°C. Abscissa, time in days after irradiation. Ordinate, peroxide value in μ mols per gram of oil.

TABLE 4

Beginning of rancidity development in irradiated meckerel oil
Dose: 1.0×10^6. Storage temp.: 25°C.

Time (hours)	Peroxide values, (μ mol./g.) at various intervals after irradiation			
	0	0.5	2	16
Control, air	2.3	2.3	2.4	3.4
Irradiated, air	2.3	3.9	4.9	6.6
Irradiated, vacuum	2.3	1.7	1.8	2.4
Irradiated, nitrogen	2.3	1.9	2.1	2.7

hour after irradiation in air at room temperature, there is already a noticeable increase in peroxide value which continues at about the same rate until 2 hours after irradiation. Between 2 and 16 hours after irradiation, the rate of peroxide formation diminishes and then continues as shown in Figure 2. Irradiation in vacuum or nitrogen causes a slight initial drop in peroxide values which tend to return to the original values after 16 hours of storage at room temperature.

The effects caused by the addition prior to irradiation of .05% propylgallate and .1% butylated hydroxyanisole are shown in Figures 3 and 4. These graphs also contain curves for cod liver oil irradiated without antioxidants as well as curves for the untreated controls, both with and without antioxidants. The development of rancidity

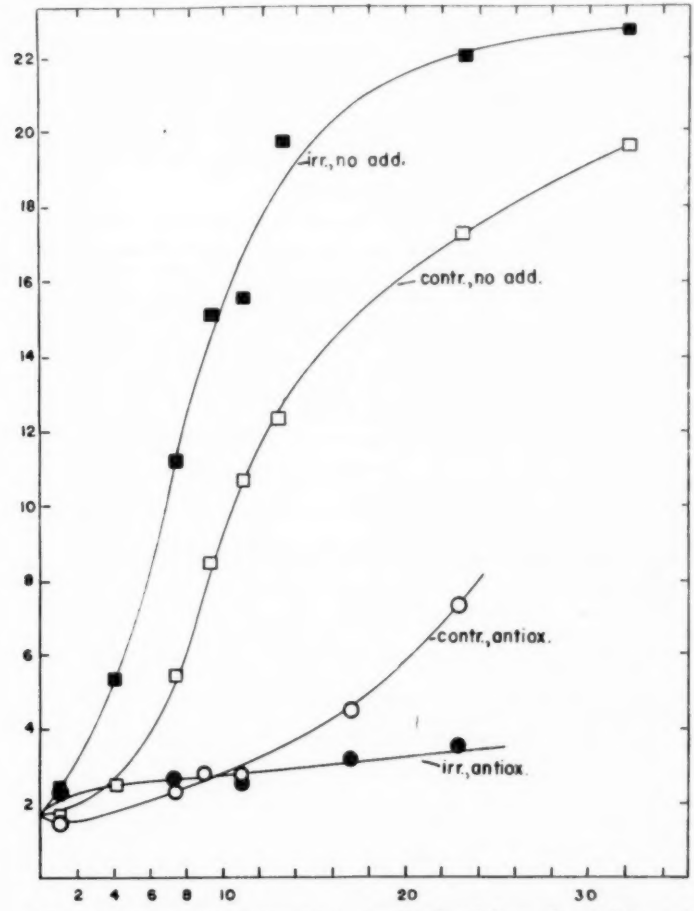

Figure 3. Development of rancidity in irradiated cod liver oil containing 0.05% propylgallate. Dose: 1.5×10^6 rep. Irradiated and stored at 25°C. Abscissa, time in days after irradiation. Ordinate, peroxide value in μ mols per gram of oil.

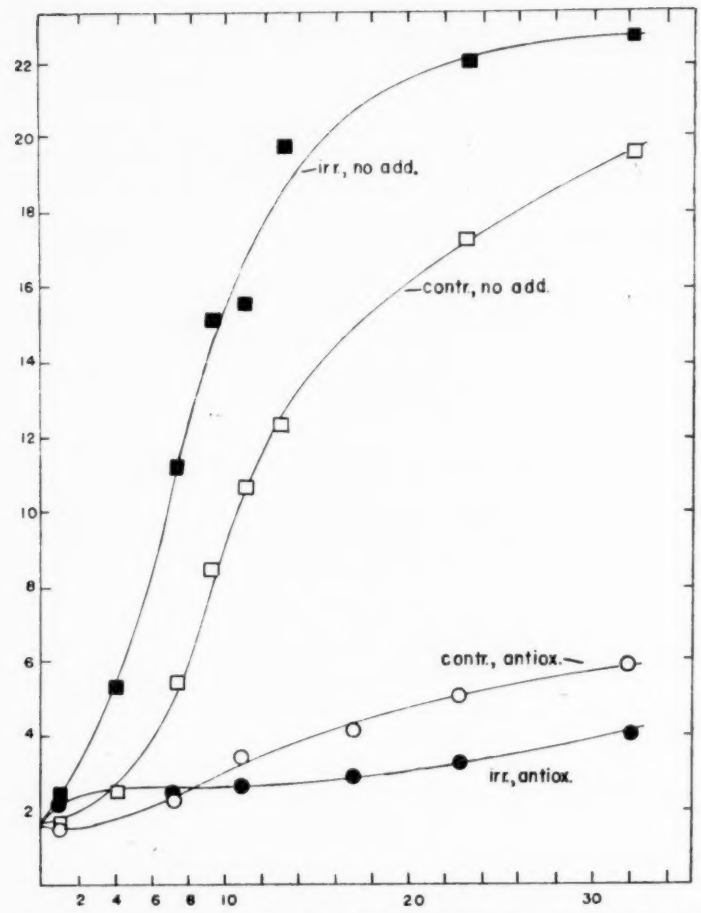

Figure 4. Development of rancidity in irradiated cod liver oil containing 0.1% butylated hydroxyanisole. Dose: 1.5×10^6 rep. Irradiated and stored at 25°C. Abscissa, time in days after irradiation. Ordinate, peroxide value in μ mols per gram of oil.

in cod liver oil containing no additives and irradiated in air follows closely that of irradiated whale oil and mackerel oil, in that the increase in peroxide values in the irradiated samples is more rapid than in the nonirradiated control; however, eventually the values of irradiated and control oils again seem to converge toward the same points. The addition of .05% propylgallate and .1% butylated hydroxyanisole affords a considerable amount of protection during the test periods, e.g. 25 to 32 days at 25°C. The retardation of peroxide formation in the irradiated cod liver oil containing propylgallate and butylated hydroxyanisole seems more pronounced than in the control samples.

It is of interest to note the steeper initial rate of peroxide formation in the irradiated oil containing antioxidants; the protective effect becomes more pronounced than in the control only after about 2 days of storage at 25°C. Propylgallate and butylated hydroxyanisole appear to be much less radiation sensitive than the naturally present protective mechanisms.

The organoleptic changes in the fish oils (Table 2) are somewhat in variance with the chemical results. Irradiation in air, regardless of temperature, causes the development of a burnt odor and a disagreeable fishy taste in both raw and refined whale oil. The magnitude of these changes, however, is reduced considerably during storage at room temperature for 6 weeks, notwithstanding the fact that the peroxide values increase considerably during this time. Cod liver oil, both common and cold cleared, undergoes similar organoleptic changes under irradiation, in that there is a development of a more fishy odor and taste; but in this instance, however, they do not decrease on storage at room temperature for 6 weeks. The addition of .05% propylgallate and .1% butylated hydroxyanisole is of no influence on the organoleptic changes, except that the antioxidants introduce, per se, an additional bitter component both in the control and in the irradiated sample. Samples irradiated in vacuum or nitrogen did not exhibit any organoleptic changes in comparison with the untreated controls, that is to say, they were indistinguishable from a freshly prepared control even after 60 days of storage at 25°C.

DISCUSSION

The results presented above indicate that the changes observed in vegetable and fish oils treated with sterilizing doses of high intensity electron bursts are very much dependent upon the individual radiation sensitivity of the oil. The decrease in unsaturation and the increase in viscosity observed in irradiated castor oil would indicate a partial polymerization. In linseed oil the changes are in viscosity and peroxide values as well as in Kreis test and acid values, thus indicating a rather complex reaction pattern. Such changes could be explained by partial polymerization[b] with simultaneous peroxide formation followed by a limited breakdown of the peroxides with the formation of lower molecular aldehydes and the liberation of additional free carboxyl groups.

The changes observed in the fish oils when irradiated, without precautions, with sterilizing doses of high intensity electrons, are much more pronounced than the ones observed under identical conditions with vegetable oils. Particularly significant is the accelerating effect of electrons on peroxide formation in fish oils irradiated in air, and it seems possible that the effect in each case is due to a destruction of naturally present protective systems. Such a mechanism is also indicated by the lack of other changes observed in the irradiated fish oils as well as by the fact that the peroxide values of irradiated and control samples eventually seem to converge towards the same end point. The initial drop in peroxide values directly after irradiation, although small, seems to occur regularly and would indicate that in addition to a destruction of natural antioxidants, the immediate effect of electron bombardment also results in a partial destruction of peroxides initially present in the oil.

That the radiation effect is rather complex is also apparent from the fact that the retardation of peroxide formation in irradiated cod liver oil containing propylgallate and butylated hydroxyanisole is more pronounced than in the control containing antioxidants. This would indicate either

[b] The polymerization effect of the radiation is presently being studied with pure, unsaturated fatty acids naturally occurring in fats and oils.

additional retention of natural antioxidants or formation of additional protective mechanisms in oils irradiated in the presence of added antioxidants. The addition of antioxidants before irradiation seems to offer a possibility to prolong the subsequent storage life of the oil and .05% propylgallate seems to be quite effective for this purpose, while .1% butylated hydroxyanisole, although effective, may cause undesirable organoleptic changes.

The accelerated initial rate of peroxide formation in irradiated cod liver oil containing added antioxidants seems, although small, to be consistent. The protective effect in the control is more pronounced than in the irradiated oils up to 2 days' storage at room temperature. After this time, however, the irradiated samples exhibit a better degree of protection. This again would indicate that one of the primary effects of electron bombardment is the destruction of naturally present antioxidants or other protective mechanisms, with the added antioxidants taking over only after an initiation period during which peroxide formation in the irradiated oils occurs at an accelerated rate. Propylgallate and butylated hydroxyanisole appear to be much less radiation sensitive than the naturally present protective mechanisms.

The unimpaired development of rancidity in fish oils irradiated in the frozen state is contrary to the beneficial effect obtained with most foods by depressing the effects on the water present. Such results emphasize the importance of water as an intermediate for indirect effects of solvent molecules causing chemical as well as organoleptic changes in irradiated foodstuffs. Freezing, e.g. conversion of a liquid into a solid system, will materially decrease indirect effects in an aqueous system. This is undoubtedly due to the reduction of radical formation, radical motility, and chain propagation. In a nonaqueous system, the absence of such mechanisms would explain the observation that peroxide formation in anhydrous oils is not diminished by irradiation at low temperatures. However, we have not been able to duplicate in fish oils the observations of Hannan and Boag on butter fat, e.g. increase of apparent peroxide values with decrease of temperature. [c]

The organoleptic changes in irradiated fish oils do not necessarily seem to run in parallel with the rancidity development as determined by peroxide values after varying periods of storage. This is in agreement with the findings by Lemon, Lips, and White *(16)*, who observed that peroxide values and iodine number determinations did not correlate highly with the flavor scores of hydrogenated linseed oil. Our observations are also in keeping with the results described by Loughlin, Riemenschneider, and Ellis *(17)*, who reported that the desirability of flavor in lards and other fats showed little, if any, relationship to peroxide value, iodine number, and free fatty acid determinations. The independence of organoleptic val-

[c] Dr. Hannan has since observed *(6)* that many of the changes in butter fat appear to be peculiar to the solid state. Irradiation and storage in this state gives rise to a marked post-irradiation increase of peroxide value and decrease of storage life which do not occur with fat molten during or immediately after irradiation. The observations with the latter system are presumably parallel to the present results with oils but the precise relationship still remains to be established.

ues from peroxide values is further brought out by the fact that none of the added antioxidants retarded the development of objectionable flavor changes due to the irradiation. Oxygen, however, must be an essential intermediate in the development of radiation off-flavors, since treatment in vacuum or under inert gases successfully inhibits the occurrence of the organoleptic changes.

These findings are parallel with our observations on the keeping qualities of whole eviscerated fish such as mackerel. Whole, fresh fish when treated to bacteriological sterility with high intensity electron bursts while packed in hermetically sealed containers, does not undergo rancidity development on prolonged storage at room temperature, even though the fish were irradiated in air. This is not necessarily in contradiction with our findings on fish oils since in whole fish, nature provides its own inert container because conditions prevailing in the oil-bearing cells are essentially anaerobic. This behavior is changed in the case of fish fillets where large cut surfaces are exposed to air and considerable impairment to cell structure has taken place. In these instances, increase in rancidity on irradiation in air is rapid but can be avoided if the radiation-sterilization is carried out in a vacuum or inert gas.

The results of Hollaender *et al. (8)* indicate that irradiation of *E. coli* B/r in an inert atmosphere requires a substantial increase in dose for a given destruction. Such an effect, if applicable to all types of organisms, would make it appear that radiation sterilization of foods in vacuo or in nitrogen would require increased doses of radiation, thus negating the possibility of a beneficial effect. It has been our experience, however *(12)*, that a dose of 1.5×10^6 rep $\pm$ 10% is satisfactory to obtain commercial sterility regardless of whether the irradiation is carried out in air, in vacuo, or in nitrogen. The apparent discrepancy between our results and those of Hollaender *et al.* may well be due to the great differences in dose rate *(4)* inherent in the 2 techniques of irradiation.[4]

Our results on mackerel oil confirm the observations of Nickerson, Goldblith, and Proctor *(18)*, who noted an increase in peroxide number in oil obtained from mackerel fillets which had previously been irradiated in air with cathode rays. However, we did not note the increase in free fatty acids observed by these authors. This may, however, be due to the fact that they isolated the mackerel oil from irradiated fillets where prior changes in the tissues could have been responsible for the increase in acidity.

SUMMARY

The effects of sterilizing doses of high intensity electron bursts on cotton seed, castor and linseed oil, as well as cod liver, herring, mackerel, and whale oil have been examined by chemical and organoleptic methods. The magnitude of the radiation effect was found to depend largely on the composition of the individual oil. The radiation initiated reaction mechan-

[4] Preliminary results *(23)* with *E. coli* B/r irradiated with high intensity electron bursts in inert gases or in the presence of chemicals such as sodium thiosulphate ($Na_2S_2O_4$) seem to confirm the observations that at very high dose rates the difference in percentage destruction for a given dose is very little dependent upon conditions of environment during the irradiation.

ism seems to be complex, including polymerization, bond breakage, as well as a variety of oxidative changes.

The accelerated peroxide formation observed in fish oils is probably due to destruction of natural antioxidants since it can be retarded in the presence of added antioxidants and by irradiation in vacuum or inert gases.

Contrary to the beneficial effects obtained with many foods, irradiation in the frozen state does not diminish or retard radiation effects in anhydrous oils.

The organoleptic changes in irradiated oils do not seem to run parallel with the chemical changes, and consequently are not influenced by the presence or absence of antioxidants. However, oxygen as well as air must play a role in the formation of radiation induced off-flavors since treatment in vacuum or under inert gases successfully inhibits the occurrence of organoleptic changes.

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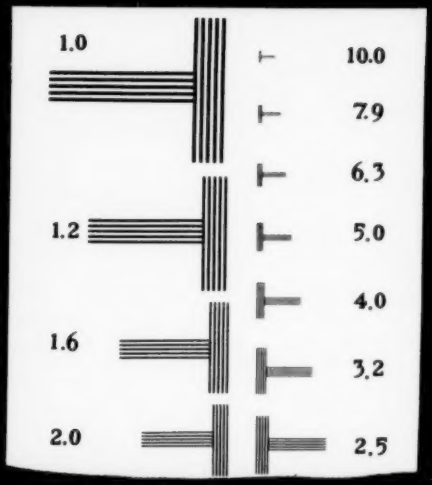

100 MILLIMETERS

INSTRUCTIONS Resolution is expressed in terms of the lines per millimeter recorded by a particular film under specified conditions. Numerals in chart indicate the number of lines per millimeter in adjacent "T-shaped" groupings.

In microfilming, it is necessary to determine the reduction ratio and multiply the number of lines in the chart by this value to find the number of lines recorded by the film. As an aid in determining the reduction ratio, the line above is 100 millimeters in length. Measuring this line in the film image and dividing the length into 100 gives the reduction ratio. Example: the line is 20 mm. long in the film image, and $100/20 = 5$.

Examine "T-shaped" line groupings in the film with microscope, and note the number adjacent to finest lines recorded sharply and distinctly. Multiply this number by the reduction factor to obtain resolving power in lines per millimeter. Example: 7.9 group of lines is clearly recorded while lines in the 10.0 group are not distinctly separated. Reduction ratio is 5, and $7.9 \times 5 = 39.5$ lines per millimeter recorded satisfactorily. $10.0 \times 5 = 50$ lines per millimeter which are not recorded satisfactorily. Under the particular conditions, maximum resolution is between 39.5 and 50 lines per millimeter.

Resolution, as measured on the film, is a test of the entire photographic system, including lens, exposure, processing, and other factors. These rarely utilize maximum resolution of the film. Vibrations during exposure, lack of critical focus, and exposures yielding very dense negatives are to be avoided.

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